AN EVALUATION OF CLINICAL PROCEDURES USED IN DENTAL IMPLANT TREATMENT IN POSTERIOR MAXILLA USING FLAPLESS TECHNIQUE

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ABSTRACT

The review of literature in the posterior maxilla areas shows that flapless surgery could be a viable and predictable treatment method for implant placement, indicating both efficacy and clinical effectiveness. The available short-term and long-term results reported in this published review illustrate that the flapless approach, initially endorsed for inexperienced clinicians, requires more skill and pre-surgical preparation than initially thought. In light of modern advances in digital imaging and computer-guided surgery, this technique can now be used with more predictability. However, caution should be exercised in using flapless surgery to minimize complications and mishaps. This implies that implant practitioners must be willing to learn and adapt to new technology. Being diligent and cautious with new techniques and technology are measures that could help to safeguard against unpleasant complications in flapless implant surgery, including those implants placed in the posterior maxilla. Hence, the application of flapless implant surgical technique in everyday procedure should be limited to experienced surgeons; it is not endorsed for inexperienced clinicians. However, further long-term clinical controlled studies are needed.

Minimal invasive surgery has transformed modern-day surgery including dental implantology, especially flapless surgery. The first study of this PhD thesis is "Flapless Dental Implant Surgery: A retrospective study of 1241 consecutive implants". The aim of this study is to identify the predictors of implant survival when using the flapless protocol in two private practices retrospectively. Results were analysed using a life table analysis, and indicated a cumulative 5- and 10-year implant

survival rate of 97.9% and 96.5% respectively. Most of the failed implants occurred in the posterior maxilla (54%), in type 4 bone (74.0%), and 55.0% failed implants occurred in smokers. This study has demonstrated that flapless dental implant surgery can produce a relatively high survival rate compared with other studies using traditional flap techniques. It has also reinforced the notion that a predictable outcome with high efficiency and efficacy can be achieved with careful treatment planning and adherence to evidence-based surgical and restorative protocols.

The success of dental implants over the last twenty years, has led to a rapidly increasing number of human and animal research projects, but no research had been done using surface-treated implants in the posterior maxilla of osteoporotic rats. The second part of this thesis is aimed at evaluating the effects of osteoporosis on osseo-integration around titanium implants following a tooth extraction in the posterior maxilla of rats. The results of this study demonstrate that with a carefully planned and executed surgical implant protocol, implant placement following extraction in the posterior maxilla can be reproducibly achieved. The bone to implant contact (BIC) and bone density (BD) measurements in the ovariectomized (OVX) group were significantly inferior to those in the SHAM group at both 28 and 56 days, which indicates that osteoporosis could reduce the amount of osseo-integration of dental implants in the posterior maxilla. These results lead to the conclusion that the surgical placement of an implant into the mesial root of a freshly extracted maxillary first molar in rats could mimic implant placement in poor quality bone such as is seen in an osteoporotic posterior maxilla. With experienced and careful surgical implant protocol, implant placement following extraction in the posterior maxilla of osteoporotic rats can produce satisfactory results, and implant insertion following

fresh extraction in poor quality bone is not an absolute contraindication. However, as compared with the SHAM group, the indices of osseo-integration (BIC and BD) in the OVX group were inferior. This finding suggests that caution needs to be exercised when placing dental implants in osteoporotic subjects. Thus, there is a need to find a new treatment modality to enhance osseo-integration in osteoporosis. This study hopes to benefit the research community, the commercial community, and members of the public, by providing a better understanding of the use of surface-treated implants in a difficult implant area - the posterior maxilla of osteoporotic patients.

The third study is aimed at evaluating the relationship between implant placement, poor quality bone, simvastatin (SIM), and osseo-integration of surface-treated implants in the posterior maxilla of osteoporotic rats. Results showed that the osseo-integration as assessed using bone formation rate (BFR), BIC and BD, in the three groups, illustrated significant differences, with SHAM > OVX+SIM > OVX group. In turn, this implied that simvastatin could promote bone mineralization in OVX rats. In conclusion, this study has shown for the first time that simvastatin can positively affect the osseo-integration indices, and successfully promote osseo-integration in the posterior maxilla in osteoporotic rats.

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LIST OF ABBREVIATIONS

ALP	Alkaline Phosphatase
ANOVA	Analysis of Variance
AUC	Area Under Curve
BALP	Bone Alkaline Phosphatase
BD	Bone Density
BFR	Bone Formation Rate
BGP	Bone Gamma-Carboxyglutamic Acid-Containing Protein
BIC	Bone to Implant Contact
BI	Bleeding Index
BMD	Bone Mineral Density
BMP-2	Bone Morphogenic Protein 2
Ca^{2+}	Calcium Ion
CAD/CAM	Computer-Aided Design/Computer-Aided Manufacturing
CBCT	Cone Beam Computed Tomography
CI	Confidence Intervals
CLSM	Confocal Laser Scanning Microscopy
CT	Computed Tomography
DEXA	Dual-Energy X-ray Absorptiometry
ELISA	Enzyme-Linked Immunosorbent Assay
Fisher's LSDT	Fisher's Least Significant Difference Test
GBR	Guided Bone Regeneration
HMG-CoA	3-Hydroxy-3-Methylglutaryl-Coenzyme A
HU	Hounsfield Units
ITI	International Team for Implantology
Md	Mandible
MIS Implant	Make It Simple Implant
ML	Attachment Level
mBI	Modified Bleeding Index
mPI	Modified Plaque Index
mRNA	Messenger RiboNucleic Acid
Mx	Maxilla
NR	Not Reported
OP	Osteoporosis
OVX	Ovariectomized
OVX+SHAM	Ovariectomized and Sham
OVX+SIM	Ovariectomized and Simvastatin
PD	Probing Depth
SD	Standard Deviation
SHAM	Sham
SIM	Simvastatin
SLA	Sand-blasted Large grit Acid-etched
SPSS	Statistical Package for the Social Sciences
STI	Surface Treated Implant
TAD	Temporary Anchorage Device
3D	Three Dimensional

TGF	Transforming Growth Factor
VAS	Visual Analogue Scale
WNT	WNT Signal transduction pathways
WKM	Width of Keratinized Mucosa

STATEMENT OF ORIGINAL AUTHORSHIP

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature: QUT Verified Signature

Date: 5 February 2014

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This thesis is dedicated to my son [Joshua], my daughter [Natasha], and my wife [Marie] for putting up with me during my PhD candidature.

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Chapter 1: INTRODUCTION

This introduction chapter delineates the background *(section 1.1)*, context, research problems and hypotheses *(section 1.2)*, and its purposes, aims and objectives *(section 1.3)*. Section 1.4 describes the significance of the research while *section 1.5* defines the scope and provides definitions of terms used. *Section 1.6* covers the report on progress including: List of Publications by Candidate *(Section 1.6.1)*, Linkage between Publications *(1.6.2)*, and their associated summary *(1.6.3)*. Finally, *section 1.7* contains an outline of the remaining chapters of the thesis.

1.1 BACKGROUND

The inauguration of osseo-integration by P. I. Branemark[1] modernized oral therapy in partially and fully edentulous patients using surgical flap technique. Dental implant rehabilitations have substantially achieved clinical and scientific recognition as a mainstream dental treatment [1]. As the implant success rate has increased and more implants are being used, dental practitioners have to contend with greater complications faced in those patients with medical problems such as osteoporosis. Thus, there is a need to search for means to deliver better outcomes in more efficient and minimally invasive approaches. Flapless surgery is one of the ways to deliver this outcome. I am a specialist oral surgeon with a strong interest in daily practice of dental implants, which has led me to undertake this thesis.

1.2 CONTEXT, RESEARCH PROBLEMS AND HYPOTHESES

The major focus of this study is on evaluating clinical procedures used in dental implant treatment in the posterior maxilla using flapless technique.

1.2.1 The research problems

The literature shows inadequate and often contradictory data regarding dental implant therapy in the reduced quality bone of the posterior maxilla. Though there is marginally lower osseo-integration success in these areas, low quality bone is not a total contraindication for dental implant therapy [2, 3]. The anatomical and structural features of the posterior maxilla, such as poor bone quality and quantity, owing to its proximity to the maxillary sinus, may compromise the clinical outcome of dental implants.

Furthermore, research has indicated that although the traditional flap implant technique has enjoyed a high success rate, this is usually coupled with initial surgical trauma and shortfalls[4, 5], especially, in the posterior maxilla of poor quality bone osteoporotic patients. There is an evident trend toward a minimally invasive surgical technique to overcome the issue of surgical trauma and gaps associated with traditional flap technique. Flapless methods offer a promising alternative to the traditional flap counterpart. Many osteoporotic dental implant patients use simvastatin to control their cholesterol. These are research problems deserving to be explored in this study.

1.2.2 Research questions

(1) How can we find out whether flapless technique is a safe, efficacious, and effective surgical technique for placement of a dental implant?

To answer this question, a systematic review of the literature is the key. There have been many publications on the subject of dental surgical flapless techniques but none has actually focussed specifically on the posterior maxilla. Being the rear part of the upper jaw, the posterior maxilla has a tendency to have broader bone width and less demand for implant aesthetics, but is usually associated with inadequate bone height and poorer bone quality.

With advances in maxillofacial imaging technology, such as three-dimensional cone beam computed tomography (CBCT), and their associated planning software, implant planning has been made easier than ever before. This is due to their precise three-dimensional data that allow the operator to simulate flapless surgery prior to the clinical operation. Thus, flapless surgery is no longer a guessing game but could be a predictable method with good training and proper diagnostic aids. This question is yet to be answered and systematic review is a method of choice to address this question.

(2) What sort of pilot study should be used to identify the key areas for future research?

Long-term randomised controlled clinical studies would have been an ideal method of study. However, the basic difficulties are time and research logistic constraints. The felt need gears toward finding alternative ways to find the answers to the above study constraints. A medium-term retrospective study of dental implants seemed to be the solution. Hence, a ten-year retrospective study of clinical procedures

used in dental implant treatment in the posterior maxilla using flapless technique was chosen.

In the past four decades, numerous studies have evaluated the results of dental implant treatment using conventional flap techniques in university settings, but there are a few publications on implants placed using flapless surgery in a private practice setting by the same dental implant practitioner. A ten-year implant study made in Brisbane [6] used flap techniques on normal patients but no study of flapless technique was reported. One objective of this study is to assess dental implant outcome using flapless technique in two private practices retrospectively by: (1) carrying out retrospective studies to assess dental implant outcomes of 1241 implants using flapless technique only in two private dental practices in Brisbane; (2) collecting the results that consist of patients' demographics, details of implants placed, and implant sites, and (3) evaluating dental implant treatment outcome to identify the predictors of implant survival using innovative flapless surgical techniques.

(3) How do medical conditions such as osteoporosis affect the outcome of flapless implant surgery in the posterior maxilla?

Osteoporosis is a medical condition that has been described as having undesirable consequences on bone formation during dental implant osseo-integration and, as such, it is regarded as a risk factor for implant failure[7, 8].

Ideally, *in vivo* human studies would be an excellent way to assess the effect of osteoporosis on dental implant osseo-integration. This would include histmorphometric evaluation. However, ethical issues compelled this study to be conducted on animals. Rats appear to be a good choice for an *in vivo* study of this type to evaluate the effects of osteoporosis on osseo-integration around titanium implants following extraction in the posterior maxilla of OVX rats.

(4) Could the commonly used cholesterol-lowering drug simvastatin help in osseo-integration of titanium dental implants in osteoporotic subjects?

Hypothetically, medications that could increase bone growth would most probably boost osseo-integration of oral implants in low density bone areas such as those presented in osteoporotic patients.

One of the usual statin products is simvastatin, which is a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor. It is frequently utilized as a cholesterol-reducing drug and hinders hepatic cholesterol biosynthesis [9, 10]. Contemporary studies have suggested a positive impact of statins on bone mineral density (BMD)[9, 11, 12]. The most credible elucidation put forward for this is that many statins enhance the mRNA manifestation of bone morphogenetic protein (BMP-2) in osteoblasts, with a subsequent rise in bone development when injected subcutaneously next to murine calvaria.[13] Also, the irregular surface-treated titanium implants such as those created in Straumann® SLA implants and its counterparts, were understood to be osseo-conductive to bone growing cells-osteoblasts via increasing mRNA manifestation of bone morphogenetic protein (BMP-2)[14]. Numerous animal and human studies have been performed to elucidate the clinical importance of statins. Consequently, statins have been proposed as potential means in the controlling of osteoporosis and as a chosen drug for this study.

1.2.3 Hypotheses

Hypothesis 1: Systematic review is an appropriate way to find out gaps in the literature regarding the implant surgical flapless research technique.

Hypothesis 2: Dental implant research using retrospective studies is a useful tool to evaluate treatment outcomes and trends.

Hypothesis 3: The maxilla is an ideal place for immediate implantation as it is easier to induce osteoporosis in the maxilla than in the mandible. It is worthwhile to carry out *in vivo* research as there has been very little work published on compromised posterior maxillae and immediate implantation using STIs.

Hypothesis 4: Simvastatin in conjunction with surface-treated implants (STIs) can enhance osseo-integration in osteoporotic rats.

1.3 AIMS

The overall aim of this thesis was to carry out studies to assess the outcome of surgical flapless technique in the posterior maxilla of osteoporotic subjects. Subsidiary aims include:

Aim 1: To review the literature on the outcome of flapless surgery for dental implants in the posterior maxilla.

Aim 2: To assess ten-year dental implant outcomes using flapless technique in two private practices retrospectively.

Aim 3: To evaluate the association between bone formation during osseo-integration of immediately placed dental implants in the posterior maxilla of ovariectomized rats.

Aim 4: To evaluate the relationship between implant placement, poor quality bone, simvastatin, and osseo-integration of surface-treated implants in the posterior maxilla of osteoporotic rats.

1.4 SIGNIFICANCE OF THE RESEARCH

A sound scientific outcome of this study would provide invaluable insight for minimally invasive implant therapy, as some of the previously published literature was unfavourable towards the technique. No credible research was done on implant surgical technique on the posterior maxilla. This seems to leave a gap of knowledge in this area. The obtainable information was appraised for short- and long-term results. Thus, the practical outcomes of the study could be used to improve the efficacy and effectiveness of surgical flapless technique in the posterior maxilla of osteoporotic subjects. Therefore, this study hopes to benefit the research community, the commercial community, and members of the public towards a better understanding of the use of flapless implant surgery in a difficult low-density bone area - the posterior maxilla of osteoporotic subjects, and of the additional benefit of simvastatin on dental implant osseo-integration in the posterior maxilla of osteoporotic patients.

1.5 SCOPE AND DEFINITIONS

This thesis searches for possible means of implementing implant surgical flapless technique to improve efficacy and effectiveness of minimally invasive technique via the application of surface-treated implants (STIs) and simvastatin in the posterior maxilla.

At first glance, the scope of this thesis appears broad; however, this study focused mainly on four aspects: (1) a systematic review the current literature; (2) a retrospective clinical study; (3) an animal study to evaluate the effect of osteoporosis on osseo-integration around titanium implants in the posterior maxilla following an extraction; and (4) an evaluation of the effects of simvastatin on osseo-integration around titanium implants in the posterior maxilla of osteoporotic rats. The scope did not embrace confirmation of the clinical techniques; rather, it was limited to clinical and scientific findings of flapless surgery in implant patients and rats. Distinctive challenges of procedural flapless surgery, such as therapeutic management, scrutinizing, and appraisal, were not particularly contemplated in this thesis.

Within the context of this thesis, definitions of the key concepts and variables used are: (1) Endosseous titanium dental implant is an artificial metallic therapeutic dental device used for replacement of a missing tooth; (2) Osseo-integration is considered as direct contact of bone surrounding an endosseous titanium dental implant without soft tissue intervention; (3) Osteoporosis is a medical condition where bone quality is quantitatively inferior to that of healthy standard young adults; (4) Posterior maxilla is defined as the back part of the upper jaw, bounded anteriorly by the upper canine teeth, posteriorly by the maxillary tuberosities, bucally by buccal mucosa, and medially by the palate.

1.6 REPORT ON RESEARCH PROGRESS

This thesis is presented for the PhD by publication. It consists of six chapters, four of which were planned as separate papers authored during and after my PhD candidature. In line with PhD thesis recommendations, these papers are contained within “as submitted” or “published” with no editing.

A record of published papers is arranged in *section 1.6.1. Section1.6.2* depicts the connection between the individual papers and how, when seen as a sole adjoining form of work, they deal with the problems/difficulties, aims and objectives of this PhD thesis.

1.6.1 List of publications by the candidate

This thesis consists of four proposed papers [one already published in an international peer-reviewed journal, a further two (Chapters 3 and 4) accepted for revision, and the last paper in process of submission], which were authored during the period of my PhD candidature. Figure 1.1 presents publication particulars.

Figure 1.1 List of publications

Chapter 2	
Paper Title:	Is flapless implant surgery a viable option in posterior maxilla? A review
Authors:	N. Doan, Z. Du, R. Crawford, P. Reher, Y. Xiao Published in *International Journal of Oral and Maxillofacial Surgery*
Status:	Int. J. Oral Maxillofac. Surg. 2012; 41: 1064-1071
Bibiographical information:	http:// eprints.qut.edu.au/
Link:	
Journal Impacted Factor:	1.835
ERA Ranking:	A
Chapter 3	
A. Conference Paper Title:	Ten Years Retrospective Study Of Dental Implant Outcomes Of 1241 Dental Implants Using Flapless Techniques In Two Private Dental Practices In Brisbane, Australia
Authors:	**N. Doan, Y. Xiao , R Crawford, P. Reher**
Conference:	International Conference of Oral and Maxillofacial Surgery (1-4 November, 2011), Santiago, Chile.
	Published (*International Journal of Oral and Maxillofacial Surgery)*
Status:	Free Papers: Implants and Dentoalveolar Surgery 1 Abstract 570
Bibiographical information:	http://www.sessionplan.com/ICOMS2011/
	www2.kenes.com/icoms2011/program/.../Printable_Program.pdf

Link:	
	1.835
	A
Journal Impacted Factor:	
ERA Ranking:	
	Flapless Implant Surgery: A retrospective study of 1241 consecutive implants
B. Paper Title:	**N. Doan, Z. Du, P. Reher, Y. Xiao**
	Manuscript was reviewed and accepted for publication with modifications
Authors:	*(International Journal of Maxillofacial Implants)*
Status:	Manuscript 3201 - Receipt - The International Journal of Oral & Maxillofacial Implants
Bibiographical information:	
	www.manuscriptmanager.com/jomi/
Link:	
Journal Impacted Factor:	1.78
ERA Ranking:	A

Chapter 4

Paper Title:	An Evaluation On The Effect Of Osteoporosis On Osseointegration Around Titanium Implants In Posterior Maxilla Following A Tooth Extraction
Authors:	**N. Doan, Z. Du, J. Xiao, W. Xia, R. Crawford, P. Reher, S. Ivanovski, F. Yan, J. Chen, Y. Xiao**
Conference:	Abstract was accepted by 22nd ASBTE Conference and was presented on 2 April 2013.
Status:	Manuscript was accepted for review by the journal, *Clinical Implant Dentistry and Related Research.* Manuscript ID is CID-13-010wwwjp.blackwellpublishing.com/bw/permis.asp?ref=0905-7161...
Bibiographical information:	
Link:	
	3.5.
Journal Impacted Factor:	A
ERA Ranking:	

Chapter 5

Paper Title:	The Effects Of Simvastatin On Osseointegration Around Titanium Implants In Posterior Maxilla Of Osteoporotic Rats
Authors:	**N. Doan, Z. Du, R. Crawford, P. Reher, S. Ivanovski, Y. Xiao**
	C17044 IHBI Inspires Postgraduate Student Conference 2012 Gold Coast,

Conference:	Qld, Australia
Status:	Abstract was accepted by IHBI Inspires 2012 and was presented on 23 November 2012
Bibiographical information:	Manuscript is in the process of submission to the *Journal of Clinical Oral Implants Research*
Link:	wwwjp.blackwellpublishing.com/bw/permis.asp?ref=0905-7161...
Journal Impacted Factor:	2.5.
ERA Ranking:	A

B. Abstract Title:	The effects Of Simvastatin On Osseointegration Around Titanium implants In Posterior Maxilla Of Osteoporotic Rats (Part II)
Authors:	**N. Doan, Z. Du, R. Crawford, P. Reher, S. Ivanovski, Y. Xiao**
Conference:	C17044 IHBI Inspires Postgraduate Student Conference 2012 Gold Coast, Qld, Australia
Status:	Accepted
Bibiographical information:	Published in IHBI Inspires Postgraduate Student Conference 2012
Link:	http://www.ihbi.qut.edu.au/about/events/postgconf_3.jsp

1.6.2 Linkage between publications

The course of acquiring an understanding of implant flapless surgery requires novel methods of scientific investigation. In order to carry out a thorough evaluation of this technique, the study should encompass systematic literature review, retrospective clinical study, and animal *in vivo* studies.

Apart from the introduction chapter, the papers illustrated in chapters 2 to 5 outline the key findings of the study. Chapter 2 summarizes the main finding and gaps in the literature; chapter 3 uses the significant findings from chapter 2 as a theme to conduct a retrospective study, as no previous flapless study had been done in a similar geographical area. The statistical results from the retrospective study were then

employed as research topics in the investigation of osseo-integration of STIs in the posterior maxilla of osteoporotic rats (chapter 4); and finally chapter 5 elucidates the effects of simvastatin on osseo-integration of STIs in the posterior maxilla of osteoporotic rats. Chapter 6 wraps up the results from the four papers.

1.6.3 Summary

The collection of papers displayed in chapters 2 to 5 of this study tackles the key research question: **how to evaluate *"the clinical procedures used in dental implant treatment in the posterior maxilla using flapless technique"?***

In particular, *chapters 2 and 3* create the main conjectural input on how implant flapless can be validated clinically; and *chapters 4 and 5* scientifically substantiate the application of flapless implant treatment on osteoporotic rats. The connections between these publications are established by the logically sequential organization of individual chapters so that its relevance to the specific question of improving clinical procedure in dental implant using flapless technique can be instituted.

1.7 THESIS OUTLINE

This thesis comprises four parts: (1) a systematic review of the literature on the posterior maxilla and the three studies, namely: (2) "Ten year outcomes of Dental Implant Flapless Surgery: A retrospective study"; (3) "An evaluation on the effect of osteoporosis on osseointegration around titanium implants in posterior maxilla following an extraction"; and (4) assessment of "The effects of simvastatin on osseointegration around titanium implants in posterior maxilla of osteoporotic rats".

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Chapter 2: Literature Review

Published in *International Journal of Oral and Maxillofacial Surgery*

Is Flapless Implant Surgery a Viable Option in Posterior Maxilla? A Review

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***Keywords**: dental implants, flapless surgery, complications, maxilla, survival, success, efficacy, effectiveness, guided surgery, review*

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Statement of Contribution of Co-Authors for Thesis by Published Paper:

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Doan N Signature  Date: 5 February 2014	• Concept and design. • Analysis and interpretation of research data; • Drafting significant parts of a work, • Critically revising it so as to contribute to the interpretation.
Du Z	• Concept and design, • Analysis and interpretation of research data; • Critically revising it so as to contribute to the interpretation
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Reher P	• Analysis and interpretation of research data; • Critically revising it so as to contribute to the interpretation.
Crawford R	• Critically revising it so as to contribute to the interpretation.

Principal Supervisor Confirmation

I have sighted email or other correspondence from all Co-authors confirming their certifying authorship

Professor Yin Xiao		5 February 2014
Name	Signature	Date

2.1 ABSTRACT

Objectives: This article is to review the literature pertinent to the outcomes of flapless surgery of dental implant in the posterior maxilla.

Data source: The literature search was carried out using as keywords: flapless, dental implants, maxilla. Hand-search and Medline search were performed on studies published between 1971 and 2011.

Study eligibility criteria: We have included research on a minimum of 15 dental implants with a follow-up period of one year, an outcome measurement of implant survival but excluding studies involving multiple simultaneous interventions and studies with missing data. The Cochrane approach for cohort studies and the criteria of the Oxford Centre for Evidence-Based Medicine were applied.

Result: Of the 56 published papers selected, 14 papers on flapless technique showed a high overall implant survival rate. The prospective studies yielded 97.01% (95% CI: 90.72 to 99.0) while retrospective studies or case series illustrated 95.08% (95% CI: 91.0 to 97.93) survival. The average of intraoperative complications was 6.55% using the flapless procedure.

Conclusions: The limited data obtained showed that flapless surgery in posterior maxilla areas could be a viable and predictable treatment method for implant placement. Flapless surgery tends to be more applicable in this area of the mouth. Further long-term clinical controlled studies are needed.

2.2 INTRODUCTION

The introduction of osseo-integration in 1977 by Per-Ingvar Brånemark[1] has revolutionized oral rehabilitation in partially and fully edentulous patients. This concept was based on the utilization of a muco-periosteal flap. The flap was designed for visualization of underlying bone by reflecting the alveolar crest soft tissue for placement, and closure with suture upon completion of the procedure. Accordingly, this concept implies that implants should be covered by soft tissue to warrant primary stabilization and decrease infection as a standard of care. Because of this, for many practitioners the flap technique has remained as the mainstay of implant surgery, as it allows better visualization, particularly in inadequate bone quantity areas, and it also provides the ability to manipulate soft tissue in the highly demanding aesthetic regions. Despite its popularity, studies also have shown that flap techniques have a number of disadvantages, including: gingival recession, bone resorption around natural teeth[15, 16], soft tissue deficiency from flap raising, and negative implant aesthetic outcomes, especially in the anterior maxilla.[5]

Over the last three decades there have been multiple modifications to implant flap design, including the flapless surgical technique. In contrast to the flap technique, implant flapless surgery does not require reflection of a muco-periosteal flap while perforating the alveolar mucosa and bone. Therefore, flapless surgery generates less postoperative bleeding, less discomfort for the patient, shorter surgery time, and reduced healing time. The patients usually heal with minor or no swelling.[17, 18] Furthermore, the flapless technique utilises either rotary burs or a tissue punch to gain access to bone without flap elevation. Consequently, the vascular supply and surrounding soft tissue are well preserved. Sclar (1999)[19] mentions that the application of flapless technique has been well established, in immediate extraction and site preservation with minimal complication. With the advance of flapless surgery the traditional flap method is being challenged. The original idea is now being perceived as unnecessarily complicated.

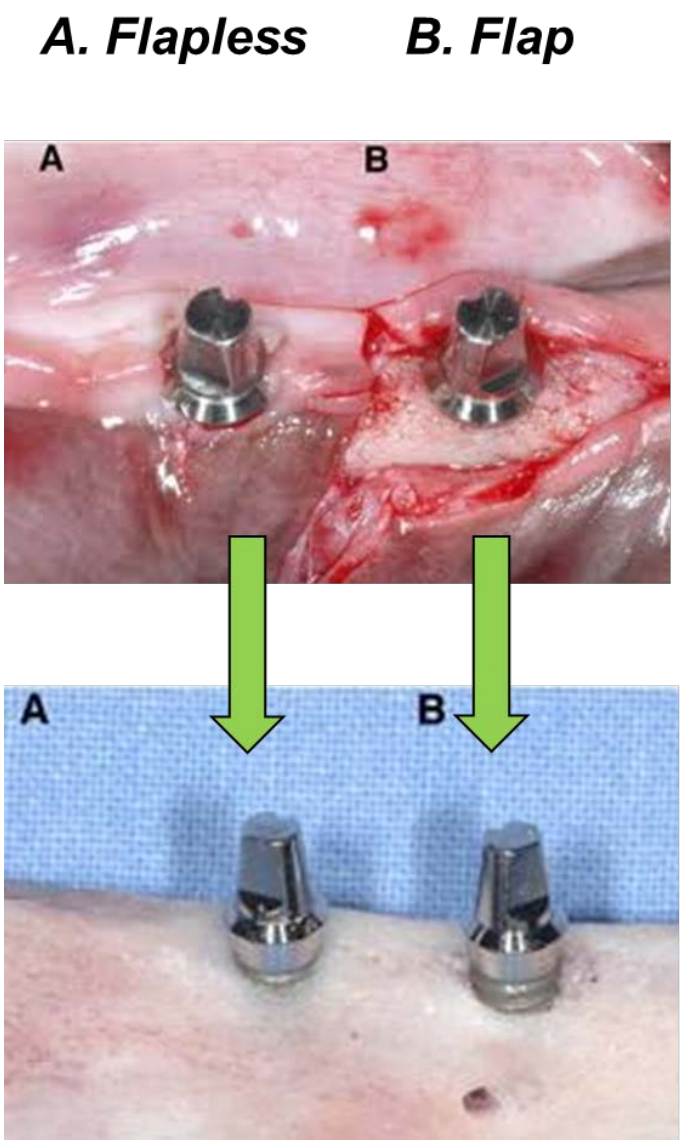


Figure 2.1 Flapless technique (A) vs. flap (B) technique. More bone resorption was observed in flap (B) than in flapless (A) (Modified from Cheong et al, 2010)

Traditionally, flapless surgery has been regarded as a technique with multiple limitations, such as: poor control of precise drilling depth owing to difficulty of observing the drilling direction of the alveolar bone; inability to preserve keratinized gingiva by a tissue punch perforation; and poor ability to assess the implant point of entry owing to the lack of direct vision of the recipient bone. Therefore, it is very difficult to correct intraoperative peri-implant defects. As this implies, flapless surgery is mainly used for cases where there is enough bone quantity and quality, as well as a sufficient quantity of keratinized gingiva.[20] It seems that posterior maxilla areas can satisfy these flapless surgery demands.

Reviewing the literature on animal studies helps one to further understand the flapless surgery-related issues and to improve flapless technique. These results include: the “biological width longitudinal dimensions at the buccal aspect were higher in the flap group than in the flapless group” on mini pigs;[21] "Flapless" surgical implantation into freshly extracted sockets did not aid in the avoidance of alveolar bone resorption and had no influence on the dimensional differences of the alveolar process after tooth extraction in contrast to the traditional implant placement using muco-periosteal flaps”;[22] and “a flapless implant surgery can be utilized for the placement of dental implants, and the application of a tissue punch larger than the diameter of the implants is not recommended, as it can endanger the result of the implantation process.” [23]

The posterior maxilla is defined as the back part of the upper jaw, bounded anteriorly by the upper canine teeth, posteriorly by the maxillary tuberosities, buccally by buccal mucosa, and medially by the palate. The supporting alveolar bone in this area is usually wide enough for implant placement, particularly in the molar region.

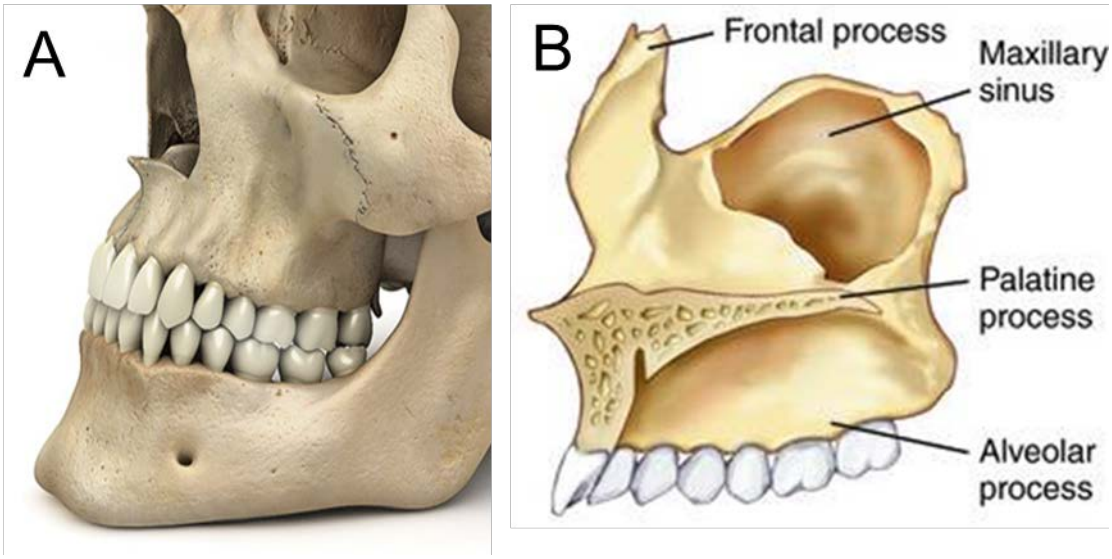


Figure 2.2 Posterior maxilla: (A) buccal aspect, and (B) palatal view (Modified from Mosby's Medical Dictionary, 8th edition. 2009, Elsevier).

Following tooth loss, resorption has been shown to adhere to a predictable pattern: the alveolar labial aspect of the ridge is the main site of resorption, which diminishes first in width and then in height.[24-26] The posterior maxilla not only has different anatomy and bone quality, but also has a distinctive buccal and palatal resorption

pattern. [24, 27] When the maxilla becomes edentulous, its shape will change according to the extent of resorption. The cortical bone in the maxilla becomes thinner and more porous posteriorly, especially the posterior maxilla in an aged population.[28] Using computed tomography, trabecular bone density varies markedly with potential implant site in the anterior (516 ± 132 Hounsfield units, HU) and posterior regions (332 ± 136 HU), which may compromise the clinical outcome of dental implants in the posterior maxilla areas. Fuh et al.[29] (2010) indicates that female maxillae also showed a smaller amount and a lower density of cancellous bone than male maxillae.

The literature also shows limited and conflicting information concerning dental implant treatment in poor quality bone of the posterior maxilla. Although there is a slightly lower osseo-integration success rate, poor quality bone is not an absolute contraindication for dental implant treatment.[2, 3, 30, 31] The anatomical and structural features of the posterior maxilla, such as poor bone quality and quantity owing to its proximity to the maxillary sinus, may compromise the clinical outcome of dental implants.

2.3 RATIONALE

In recent years, the advance of three-dimensional (3-D) dental imaging, particularly cone beam computed tomography (CBCT), and the associated planning software, used in conjunction with computer-generated surgical guides, has allowed more efficient results when using flapless techniques. This has turned flapless implant surgery into a predictable procedure with high success rates if patients are appropriately selected and an appropriate width of bone is available for implant placement.[15, 32] However, these studies mainly focus on anterior areas in order to improve aesthetic results, to shorten surgery time, and to decrease morbidity after surgery. Few studies focus on the posterior areas, especially posterior maxilla areas. There are some attributes for the flapless surgery in this area, which include: a) less aesthetic demand; b) a bone resorption pattern resulting in greater width and less undercut; c) minimal concern about nerve damage. According to these characters of posterior maxilla areas, flapless surgery appears to have more application in the posterior maxilla than in any other area. The posterior maxillae inherit some disadvantages too: lower bone density, the position of the sinus, and difficult access to the mouth. Consequently, the posterior maxilla has been described as the most difficult and problematic intraoral area

confronting the implant practitioner, which may influence the results of flapless surgery.

2.4 OBJECTIVES

This article reviews the contemporary literature pertinent to the outcome (efficiency and effectiveness) and surgical challenges of flapless dental implant surgery in the posterior maxilla, and it indicates that flapless implant surgery is an acceptable technique in the posterior maxilla.

2.5 MATERIALS AND METHODS

2.5.1 Protocol

Within the context of this review, a data search for English-language publications was carried out, using Medline search of dental journals from 1971 to 2011. Furthermore, major journals on dental implant, such as oral and maxillofacial surgery, dental implant, prosthodontics, and periodontics of a similar period were hand-searched to find relevant references. Reference search was conducted using a mixture of keywords: *dental implants, flapless surgery, complications, maxilla, survival, success, efficacy, effectiveness, guided surgery, review.*

2.5.2 Eligibility criteria, information sources, and search

Clinical human studies published in English in the last 40 years between 1971 and 2011, through either Medline search or hand-search, were categorized by study types (case report, preclinical, cohort, control clinical trial, review, and meta-analysis), and subjects (human versus animal). This review included only human-related studies of the posterior maxilla with 15 or more implants, while expert and clinical opinion publications were excluded. In order to apply the best available evidence gained from the scientific method to clinical decision making, the criteria of the Oxford Centre for Evidence-Based Medicine[4] were used to weight level of evidence in clinical studies.

In this review, “conventional implant surgery” refers to surgical techniques that require elevation of a muco-periosteal *flap* for the purpose of the implant surgery and implant placement. “Flapless implant surgery” refers to a surgical technique utilized to prepare the implant drilling site for surgical placement of the implant without the need of raising a muco-periosteal flap.

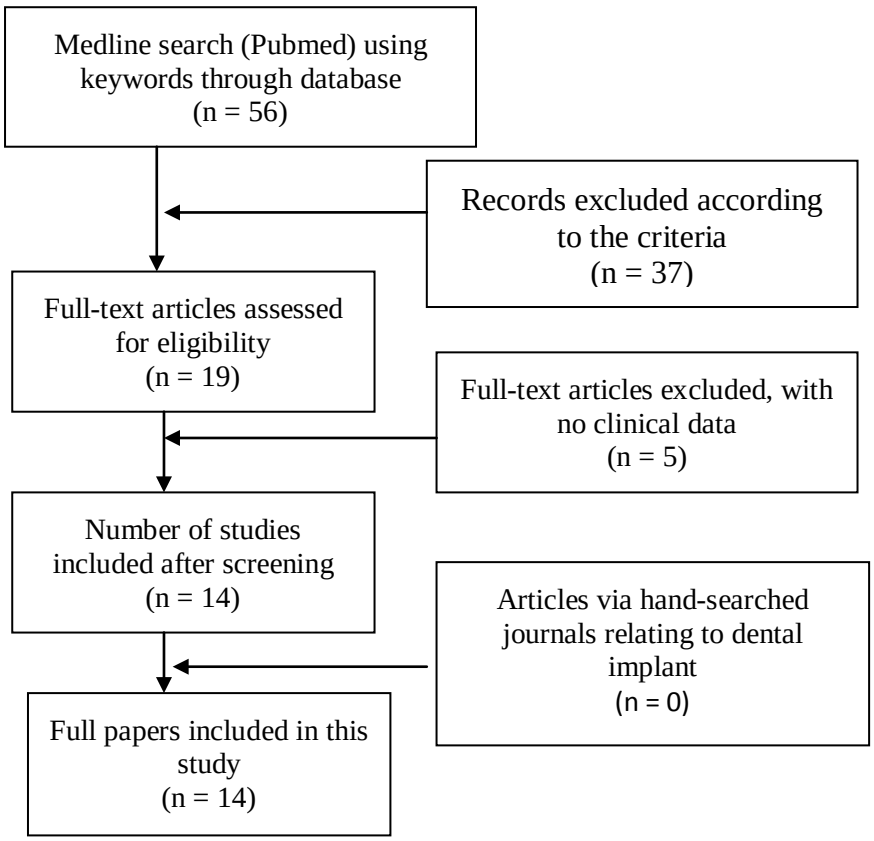
2.5.3 Data collection process and data items

The resultant information was organised into a system of comparison in a table format similar to those suggested by Brodala (2009)[5]. It contains: type of study, number of participants, dropouts, follow-up time, mean age, implant case, outcomes (survival), complications, and failures. It was assumed that these patients had no adverse medical conditions.

2.5.4 Statistical data management

The data retrieved from selected papers was managed according to the following methods: The mean values of the survival rates were case weighted to give an even distribution. Ideally, one would limit the sample to studies that use Kaplan-Meier methods to estimate survival and include those results of the few studies that meet the new inclusion criteria. However, owing to the limited number of publications available, retrospective studies were included in the review. Nevertheless, in order to improve the validity of these studies, the classification of levels of evidence of Oxford Centre for Evidence-Based Medicine (2001) was used. Furthermore, the studies were organized, reported, and then stratified by the levels of evidence.

Figure 2.3 Flowchart of article selection for review



2.6 RESULTS

2.6.1 Medline search results

The Medline search found 56 articles; 19 studies were deemed to meet the inclusion criteria. Of these, 5 systematic reviews were excluded as they did not involve clinical studies (Figure 1). According to the classification of Oxford Centre for Evidence-Based Medicine (2001), of the 14 remaining papers (table 1, 2 and 3): 6 were level 2 prospective cohort studies; 5 were retrospective; one was a non-randomized trial; and two were case studies (level 4) that looked at implant success and survival rate as well as other clinical variables. Of these, 10 were short-term studies (6 days) that evaluated intraoperative complications such as morbidity and comfort (level 2). The only long-term clinical study (level 2) was a retrospective cohort study. The most common features encountered in these studies were the use of guided surgical techniques during the course of their treatment planning. The differences in study designs were treatment in a single location versus multiple locations of the maxilla.

2.6.2 Implant Survival Rate

Life time analysis was calculated using Kaplan-Meier methods to estimate survival[33]. The survivals obtained were then case weighed. In a long-term study of implant outcomes[34] consisting of 778 patients and 2,040 implants over a mean studied period of 19 months, the results indicated fairly high survival rate. The prospective studies yielded 97.01% (95% CI: 90.72 to 99.0), while retrospective or case series evaluation showed 95.08% survival (95% CI: 91.0 to 97.93). These results illustrated clinical efficacy with prospective studies and clinical effectiveness respectively.

There were three studies[35-37] that used flapless method in conjunction with navigated surgical protocols, and the authors reported a survival rate ranging from 87.3% to 97.8%. The authors concluded that navigated surgical technique might not be appropriate for all types of bone morphology, but could be a viable and predictable treatment modality. Some complications arise during treatment. As such, this technique could be sensitive to the operator's experience and progress on the learning curve.

Table 2.1 Demographics of studies

No	Authors	Study design and Oxford Evidence based level	No of Patients	No drop outs	Remaining Implants after dropouts	No remaining Implants after dropouts of terminal events	Age range (Years)	Mean age (Years)	Follow up period (Months)	Success rate (%)	Mean survival rate (%)
1	Rocci et al. (2003)[5]	Prospective cohort (Level 2)	46	0	97	9	24-77	51	36	91 (prosthetic load) 94 (splint)	90.72
2	Becker et al. (2005)[23]	Prospective cohort (Level 2)	57	0	79	1	24-86	NR	24	98.7	98.7
3	van Steenberghe et al. (2005)[8]	Prospective cohort (Level 2)	27	3	164	NR	34-89	63	12	NR	NR
4	Fortin et al. (2006)[24]	Prospective comparative cohort (Level 2)	60	0	152	NR	19-82	NR	6 days	NR	NR
5	Cannizzaro et al. (2007)[25]	Prospective cohort (Level 2)	35	0	202	2	39-70	56.6	12	99	99
6	Malo et al. (2007)[26]	Prospective cohort (Level 2)	23	0	92	0	NR	NR	21	98 (Max) 97(Md)	98
7	Campelo et al. (2002)[27]	Retrospective study (Level 4)	377	18	770	37	27-83	54.7	60	(1) 97.4 (2) 100	95.25
8	Sanna et al. (2007)[28]	Retrospective study (Level 4)	30	4	183	9	38-74	56	36	98.9 (non smoker) 81.2 (smoker)	91.5
9	Sennerby et al. (2008)[29]	Retrospective study (Level 4)	43	0	117	6	NR	50	18	94.87	94.87

10	Katsoulis, J, et al. (2009)[30]	Retrospective study (Level 4)	28	0	112	NR	NR	NR	NR	NR	NR
11	Pomares Puig, C. (2010)[31]	Retrospective study (Level 4)	195	0	194	4	35-84	59.5	12	07.95	97.95
12	Oh et al. (2006)[32]	Non randomized trial (level 4)	24	0	24	NR	25-72	45	6	NR	NR
13	Merli, M, et al. (2008)[33]	Case series (level 4)	13	0	89	5	NR	NR	8	NR	NR
14	Ozan et al. (2007)[34]	Case series (Level 4)	5	0	14	1	NR	NR	14	98.3	98.3

Table 2.1 Demographics of studies included: Authors; number of patients; number of dropouts; remaining implants after dropouts; number of remaining implants after dropouts of terminal events; age range (years); mean age (years); follow-up period (months); success rate (%); and mean survival rate (%).

Table 2.2 Study Types and Results

No	Authors	No Single Tooth	Partial Edentulism	Complete Edentulism	Maxilla & Implants	Mandible & Implants	Result I	Result II
1	Becker et al. (2005)[23]	NR	NR	NR	47	32	No significant changes in PD, BI from 1 to 6.5 months	Bone loss = 0.07 mm detected by X-rays was not significant
2	Campelo et al. (2002)[27]	NR	NR	NR	282	488	Overall implant failure: 37 (4.8%) over 10 y	Analgesics were not used: 90%
3	Cannizzaro et al. (2007)[25]	0	0	**33**	33	0	Reported pain: none–slight (79%), moderate–severe (21%)	Swelling post surgically: none–slight (58%), moderate–severe 42%
4	Fortin et al. (2006)[24]	NR	NR	NR	NR	NR	Reported pain (VAS): significantly less and of shorter duration in flapless group, significantly less use of analgesics with flapless technique	NR
5	Malo et al. (2007)[35, 36]	0	0	23	18	5	Overall implant survival = 98%;	Bone loss detected by X-rays = 1.9 mm at 12

							maxilla 97%; mandible 100%	mo
6	Oh et al. (2006)[32]	24	0	0	24	0	Tendency for Papillary Index (PPI) to increase over 6 months	No differences in ML, PD, mPI, mBI, WKM
7	Ozan et al. (2007)[34]	NR	NR	NR	Yes	Yes	Total of 5 out of 5 implants survived at average 9 months	NR
8	Rocci et al. (2003)[5]	27	70	0	97	0	Implant cumulative survival rate: 91% at 36 mo	
9	Sanna et al. (2007)[28]	0	0	30	26	4	bone loss detected by X-rays at 4 y: 2.64 mm smokers; 1.3 mm non-smokers	Implant cumulative survival rate 91.5% over 66 mo
10	Sennerby et al. (2008)[29]	18	99	0	45	72	53% of implants had > 2 mm radiographic bone loss at ;> 3 mm radiographic bone loss at 37% implants	NR
11	van Steenberghe et al. (2005)[8]	0	0	27	27	0	bone loss 1.2 mm detected by X-rays at 12 months	NR
12	Pomares Puig, C. (2010)[31]	0	0	30	128	67	4 implants out of 195 failed in 3 patients during the healing period: 2 in the maxilla and 2 in the mandible. 3 of them were successfully replaced.	After one year of loading, there were no dropouts and no failure of the definitive prosthesis 12
13	Katsoulis, J, et al. (2009)[32]	0	0	40	184	0	28 patients (70%) with sufficient bone and 4 implants each (112 implants); 12 patients each have 6 implants (72 implants)	6 had insufficient bone to place any implant and 6 had combined sufficient bone and insufficient bone.
14	Merli, M, et al. (2008)[31]	0	0	28	112	0	13 consecutive patients with atrophic maxillae were treated with 89 implants (6 to 8 implants per patient)	5 implants failed in 4 patients. 1 patient dropped out. 8 months after loading, all prostheses were successful. 11 out of 12 patients reported

								their quality of life and lifestyle had improved.

Table 2.2 Study Types and Results consisted of: authors; number with single tooth; partial edentulism; complete edentulism; maxilla and implants; mandible and implants; result I; and result II. PD = probing depth; BI = bleeding index; ML = attachment level; mPI = modified plaque index; mBI = modified bleeding index; AUC = area under curve; WKM = width of keratinized mucosa; NR = not reported (modified from Bodala, 2009).

Table 2.3 Complications and Failures

No	Authors	Intraoperative Complication	Postoperative complication	No of failed implants	No of complications		
					Biological	**Technical**	**Aesthetic**
1	Becker et al. 2005[23]	NR	NR	1	0	NR	NR
2	Campelo et al. [27]	36 perforation (21 fenestrations 15 dehiscences)	NR	37	NR	NR	NR
3	Cannizzaro et al. (2007)[25]	1 perforation 1 treatment aborted	NR	2	5 (No of intermittent pain = 1, hyperplastic tissue = 1, peri-implant mucositis = 1, peri-implant peri-implantitis = 2); all < 10 months and resolved	10 (Not related to flapless placement)	NR
4	Fortin et al. (2006)[24]	NR	NR	0	NR	NR	NR
5	Malo et al. 2007[26]	NR	0	2	0	8 (fracture of acrylic denture)	NR
6	Oh et al. 2006[32]	NR	NR	3	NR	NR	2 (patient subjective; patient chose to have prosthetic redone)

7	Ozan et al. 2007[34]	NR	NR	NR	NR	NR	NR
8	Rocci et al. 2003[5]	NR	NR	9	NR	NR	NR
9	Sanna et al. 2007[28]	NR	NR	9	NR	NR	NR
10	Sennerby et al. 2008[29]	NR	NR	6	6 (same failed; immediate loading with flapless)	NR	NR
11	van Steenberghe et al. 2005[8]	NR	Marginal fistula (1) resolved	0	4 (inflamed hyperplastic gingiva)	4 (2 occlusal material fracture, resolved 1 screw loosening, 1 patient decided to change for different prosthesis)	NR
12	Pomares Puig, C. (2010)[29]	3 patients had surgical templates fracture during implantation	4 implants out of 195 failed in 3 patients during the healing period: 2 in the maxilla, and 2 in the mandible. 3 of them were successfully replaced	4	4 failed but only 3 successfully replaced. 3 patients have to be treated with antibiotics.	one patient, a new impression had to be taken to fit the provisional prosthesis	NR
13	Katsoulis, J, et al. (2009)[30]	NR	NR	0	21 out of 28 patients had exhibited a combination of sufficient or insufficient bone	NR	NR
14	Merli, M, et al. (2008)[31]	Two flaps had to be elevated in 2 patients. One template fractured during surgery.	One patient dropped out, for financial reasons, with the provisional prosthesis still in function.	5 implants failed in 4 patients.	Two flaps had to be elevated in 2 patients	One template fractured during surgery	NR

Table 2.3 Complications and Failures comprised: authors; number of intraoperative complications; post-operative complications; number of failed implants; and number of complications.

2.6.3 Post-surgical Trauma Discomfort

The level of intraoperative flapless surgical trauma was found to be minimal.[41, 46, 47] In the majority of these studies objective short-term evaluation of postoperative

complications were very limited or often omitted. One study was a comparative prospective non-randomized pilot study [40] of patients' morbidity (such as pain and facial swelling) from 1 to 7 days postsurgical using the visual analogue scale (VAS) for either flap or flapless surgical technique. The authors used 3-dimensional imaging to plan the procedures, and questionnaires were used to record feedback. All patients in the study had edentulous maxillae and each received 6 implants using relevant techniques. The authors found that the flapless surgery decreased the level of pain and postoperative swelling significantly ($P < .05$).

In a similar study using VAS as an assessing tool, the researchers [39] evaluated the level of postsurgical discomfort from day 1 to day 6 and the use of pain-killers in flap and flapless techniques. The patients in the flapless group experienced significantly reduced pain and used less analgesics than the flap counterpart ($p<0.01$).

2.6.4 Peri-implant Bone Loss

X-ray assessment of peri-implant alveolar bone loss over 12 months varied from 0.7 mm to 2.6 mm in 6 studies. [20, 41] Of the included studies, the peri-implant bone was assessed using different techniques consisting of peri-apical X-rays, panoramic radiograph, cone beam computed tomography, and radiographic fractal analysis. Minimal bone changes during a short time can be monitored using digital intra-oral radiography. In addition, radiographic fractal analysis did not appear to match histological fractal analysis, and CBCT was not consistent for bone density measures, but might have potential in structural investigation of trabecular bone[48]. Hence, periapical and panoramic X-rays were the main method for detecting bone loss. All the implants used in the studies were immediately loaded, and two studies did not use guided surgery.

Sanna et al.[42] evaluated annual bone loss of 7 non-smokers and 13 smokers after flapless implant placement using guided surgery. The authors did not report any substantial differences with regard to the mean marginal bone levels between the two groups at baseline and after a 1-year follow-up: non-smokers-baseline 0.1 mm (SD 0.5 mm), 1 year-0.8 mm (SD 1.1 mm); and smokers-baseline 0.1 mm (SD 0.4 mm), 1 year - 1.1 mm (SD 1.4 mm).

In a study of 109 Noble Biocare® one-piece implants, using flapless technique, the authors[43] showed a mean marginal bone loss of 3 mm (SD 1.4). Of these, 14%

experienced greater than 3 mm and 27% had more than 2 mm of bone loss. Furthermore, the authors found more bone loss in dental implants placed with flapless than with flap technique. The poor result yielded above was attributed to the attempt by the authors to increase efficacy and effectiveness by using one-piece implants, flapless technique, and immediate loading.

2.6.5 Post-operative Soft Tissue Responses

The effect of flapless surgery on soft tissue changes is well demonstrated in a study [38] of 79 implants using flapless technique and delayed loading, where baseline probing depths up to 1 month after insertion of final prostheses were recorded. The results showed no significant differences between the baseline (2.2 mm, SD 0.9) and up to one month (2.3 mm, SD 0.8). Certainly, long-term study of this area would be required.

Other soft tissue changes via flapless approach were investigated by a short-term study,[44] in which 25 patients were randomly assigned either to the immediate (baseline) or the delayed loading group (after 4 months). The assessing parameters were modified plaque index, modified bleeding index, probing depths, and keratinized gingiva width. No significant changes were observed at two time lines (base line and at 6 months). Here again, longer study is required to validate the results.

2.6.6 Complications

To clarify the overlapping definitions, “complication” within the context of this study is defined as unfavourable outcomes requiring chair-side assistance intra-operatively or postoperatively. Intraoperative complications range from perforations of bony plates to poor primary stability, whereas postoperative complications include technical, biological, and aesthetic complications. Technical complications include mechanical failures; biological complications encompass problems with osseo-integration, pain, infection; and aesthetic complications are poor gingival showing and unattractive prostheses.

Intraoperative complications from using flapless methods were reported in the five included studies.[34, 40] These complications range from perforations of bony plates to poor primary stability. One of the five studies[21] reported that 3.8% complications occurred during surgical procedures which caused the clinician to abandon or submerge the implant in these situations.

In a ten-year study, [34] 770 dental implants were placed via flapless techniques, in both edentulous and partially edentulous patients. The diagnostic imaging technique used was either computed tomography or two-dimensional radiography. Although surgical stents were routinely used, computed guided surgical stent was not mentioned. The authors reported 21 fenestrations for which the proposed treatment was changed to implant guided bone regeneration. In 15 instances where dehiscence occurred, the authors either chose a different implant location or totally aborted the procedure, resuming implant placement 3 months later after proper wound healing.

Of the 16 included studies, only 4 reported perforation as a complication[4]. This being the case, the complication report should be viewed with care.

2.7 DISCUSSION

As with any type of implant surgery, complications associated with flapless technique may be intraoperative, postoperative, or delayed (see Table 3). Of these, immediate postoperative complications related to flapless approach were found in two studies,[34, 40] which is minimal in comparison with the flap technique. These studies indicated that 3.8% of intraoperative surgical complications related to perforation of the buccal or lingual bony plates. Unfortunately, the bulk of the complications were found in just five studies.[8,22,29,30,31]

Additionally, the majority of the studies include no report on the presence or absence of perforations. Consequently, the effect that perforations may have upon implant survival or manifestation of complications is not clearly defined. As in conventional approaches, the postoperative and delayed complications seem to be similar. Most papers reported which jaw the implant was placed in but did not specify the exact location of the implant. Therefore, the upper posterior implants were grouped under the heading of maxilla implants, and only two studies[35, 46, 47] illustrated the success rates between maxilla and mandible (Table 2). Thus, the overall results of implants placed in the posterior maxilla using flapless technique are not clearly defined in these selected studies.

One major limitation in this paper is that the flapless surgical procedures for implant placement were used in various clinical settings. Even though there were

reports on the use of navigation and 3-D guided surgery in standard surgical procedures, there was no clear indication on the use of a surgical stent or guide.

Correct application of flapless surgery can help to lessen the morbidity and operating time, especially in the posterior maxilla. However, with inadequate planning and improper placement, flapless surgery usually ends up with unsatisfactory outcomes owing to inappropriate implant position. Therefore, the implant clinician should resort to measures that can be employed to reduce complications in flapless surgery, including the use of advanced three-dimensional (3-D) dental imaging, such as cone beam computed tomography (CBCT), and their associated planning software, used in conjunction with computer generated surgical guides. These have allowed better pre-treatment planning of flapless techniques to become more efficient and to avoid any hidden unfavourable anatomical or pathological pitfalls. This has made flapless implant surgery a predictable procedure with high success rates, provided patients are appropriately selected and appropriate bone quantity and quality are available for implant placement.[2, 19] Last but not least, thoroughness in treatment planning and experience of the operator are of paramount importance in reducing complications.

As in any new technological advance, the user has to go through a studying period to perfect the technology. This usually brings with it nuances which must be comprehended in order to avoid complications linked to the pertinent technology. For instance, there is a real danger of misreading CT information or incorrectly placing a surgical stent in implant surgery, which can cause irreparable damage. It is important that the implantologist should be dedicated to acquiring all aspects of the new technology, and should use it at a basic stage prior to advancing into complex scenarios such as guided implant placement. Therefore, the implant practitioner should be diligent, adaptable, and cautious with new technique and technology, in order to safeguard against unforeseen complications in implant dentistry.

During compilation of this review, consideration was given to author bias and other sources of bias (such as conflict of interest) in the selected studies. The Cochrane review approach was used for the appraisal of the validity of the studies involved. In addition, this paper stressed the possibility of bias in their outcomes, i.e. the chance that authors will overestimate or underestimate the actual intervention power. Furthermore, the authors were aware of study-specific matters that may cause concern about the

possibility of bias, and attempted to express judgements about these under this field of the tool.

Several tools are available for assessing the procedural value of clinical trial studies. The utilization of measures producing a summary score is not recommended. Instead, this study employed Cochrane Collaboration, the recommended specific tool for assessing risk of bias in each included study. This comprises a judgment and a support for the finding for each entry in a 'Risk of bias' table, where each entry addresses a specific feature of the study. The judgment for each item encompasses evaluating the risk of bias as 'low risk', 'high risk', or 'unclear risk', with the third category representing either deficiency of data or doubt over the potential for bias. For example, the prosthetic implant loading protocols differed vastly among the 14 studies, since loading could be immediate, delayed, or conventional. Taking into account these issues, in conjunction with additional confounding factors, implies that the outcome of any particular procedure is not 100% clear, or has unclear risk. This means that it is best to consider the above-mentioned factors prior to extrapolating any clinical outcomes. Finally, in order to correctly assess the value of the flapless approach, the researcher would require more studies of comparable loading procedures that fairly relate conventional approaches to a flapless technique.

In conclusion, the current data obtained from posterior maxilla areas showed that flapless surgery could be a viable and predictable treatment method for implant placement, indicating both efficacy and clinical effectiveness. The available short-term and long-term results reported in this review illustrate that the flapless approach, initially endorsed for inexperienced clinicians, requires more skill and pre-surgical preparation than initially thought. Moreover, the flapless approach requires greater knowledge and skill than the conventional surgical techniques. In light of modern advancements in digital imaging and computer-guided surgery, the above requirement is no longer mandatory. However, caution should still be exercised in using flapless surgery to minimize complications and mishaps. This implies that implant practitioners must be willing to learn and adapt to new technology. Being diligent and cautious with new technique and technology are measures that could help to safeguard against unpleasant complications in flapless implant surgery, including those implants placed in the posterior maxilla. Hence, the application of flapless implant surgical technique in

everyday procedure should be limited to experienced surgeons; it is not endorsed for inexperienced clinicians.

2.8 FUNDING

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2.9 REFERENCES

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Chapter 3: Flapless Implant Surgery: A retrospective study of 1241 consecutive implants

Accepted and reviewed by the *International Journal of Oral and Maxillofacial Implants* for publication

Flapless Dental Implant Surgery: A retrospective study of 1241 consecutive implants placed over 10 years

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Chapter 4: An Evaluation on the Effect of Osteoporosis on Osseointegration around Titanium Implants in Posterior Maxilla Following a Tooth Extraction (Part I)

Submitted and reviewed by the journal, Clinical Implant Dentistry and Related Research

The effect of osteoporosis on osseointegration around titanium implants in posterior maxilla following a tooth extraction

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4.1 ABSTRACT

Background: The surgical placement of implants into maxillary extraction sites in ovariectomized (OVX) rats could mimic implant placement in compromised bone in humans, such as is seen in osteoporosis.

Purpose: This study was designed to evaluate the effects of osteoporosis on osseo-integration around titanium implants following extraction in the posterior maxilla of OVX rats.

Materials and Methods: Forty-four 3 month old female Sprague-Dawley rats were used in this study. The rats were randomly divided into two groups: Sham-operated group (SHAM; n=22) and ovariectomized group (OVX; n=22). Surface-treated screw-shaped titanium implants were immediately inserted into the mesial extraction sites of the first molar in the posterior maxilla following tooth extraction. The animals were sacrificed at either 28 or 56 days post-surgery, and undecalcified tissue sections were processed for histological analysis. Bone-to-implant contact (BIC) and bone density (BD) were evaluated**.**

Results: With a carefully planned and executed surgical implant protocol, implant placement following extraction in the posterior maxilla can produce reproducible results. The BIC and BD in the OVX group were significantly inferior to those in the SHAM group at both 28 and 56 days, which indicated that osteoporosis could reduce the amount of osseo-integration of dental implants in the posterior maxilla.

Conclusion: This study demonstrated that osseo-integration (BIC and BD) was inferior in implants following extraction in the posterior maxilla of OVX rats, especially in the early healing period, indicating that lower local bone quality in OVX rats can reduce early bone formation on implant surfaces.

4.2 KEYWORDS

- Surface treated implants
- Osseo-integration
- Posterior maxilla
- Immediate implant**,**
- Osteoporosis
- Rats

4.3 INTRODUCTION

With the increasing utilisation of dental implant therapy, clinicians are faced with increasing challenges arising from compromised clinical scenarios encountered in those patients with medical conditions such as osteoporosis. While there is a marginally decreased osseo-integration success level, poor quality bone is not a complete contraindication for dental implant therapy [1-4]. The literature indicates incomplete and contradictory evidence regarding dental implant therapy in poor quality bone.[5, 6] In osteoporotic animals it has been found that unfavourable outcomes in bone healing during dental implant osseo-integration were detected, and accordingly it has been proposed that osteoporosis may be considered a potential risk factor for implant failure.[7-11] Several remedial methods have been tested for the enhancement of osseo-integration of dental implants in osteoporotic animals.[12-15]

The anatomical and structural topography of the posterior maxilla, such as inferior bone quantity after tooth loss, particularly in the vicinity of the maxillary sinus, may lower the clinical result of dental implants. Especially when one takes into account that further reduction of bone quality and quantity occurs following extraction, it is worth considering the rationale of immediate implant placement in the posterior maxilla in order to utilise existing bone at the time of extraction.

The aim of this study is to evaluate bone formation during osseo-integration of immediately placed dental implants in the posterior maxilla of ovariectomized (OVX) rats. The working hypotheses for choosing immediate implantation in the posterior maxilla are: (a) when there is adequate bone quantity, the posterior maxilla is an ideal place for immediate implantation; (b) generally, in humans and rats, it is easier to induce osteoporotic bone in the maxilla than in the mandible; and (c) it is worthwhile to carry out an *in vivo* study on the compromised posterior maxilla and immediate implantation, as there is little published work in this area.

4.4 MATERIALS AND METHODS

4.4.1 Experimental Design

The study was carried out following a protocol endorsed by the Animal Care and Use Committee of Fujian Medical University by our collaborators at Fujian Medical University. Similar research methods have been used previously.[3, 16] A total of 44 female Sprague-Dawley rats (3-month-old, SLAC Laboratory Animal Co. Ltd, Shanghai, China) were segregated into two groups with each group containing 22 rats. From each group, two rats were used for initial verification of the osteoporosis model via the use of micro CT, and 10 rats were used for dual-energy X-ray absorptiometry analysis (DEXA), which is one of the most commonly used method for gauging bone mineral density (BMD)[17], especially in osteoporosis. Using a randomized group design, animals were allocated to one of two study groups, which were labelled as sham-operated (SHAM, n= 20) and OVX (n = 20). In the OVX group, both sides of the ovaries were uncovered and totally removed by an abdominal dissection procedure. For the SHAM group, the ovaries were opened and an equal amount of adipose tissue was removed from the vicinity of each ovary. Subsequently, the fascia and skin were approximated and sutured. Commercial laboratory rat chow (Experimental Animal Centre of Zhejiang University, China) and water were accessible *ad libitum*. Osteoporosis status was confirmed by sacrificing two rats from each group and histologically analysing spine and cranial bone tissue, as well as uterine horns 56 days after ovariectomy. These methods are similar to those documented previously[3, 16]. Further, bones from the spine and cranium of 10 animals from each group were evaluated by DEXA to determine their osteoporosis status.

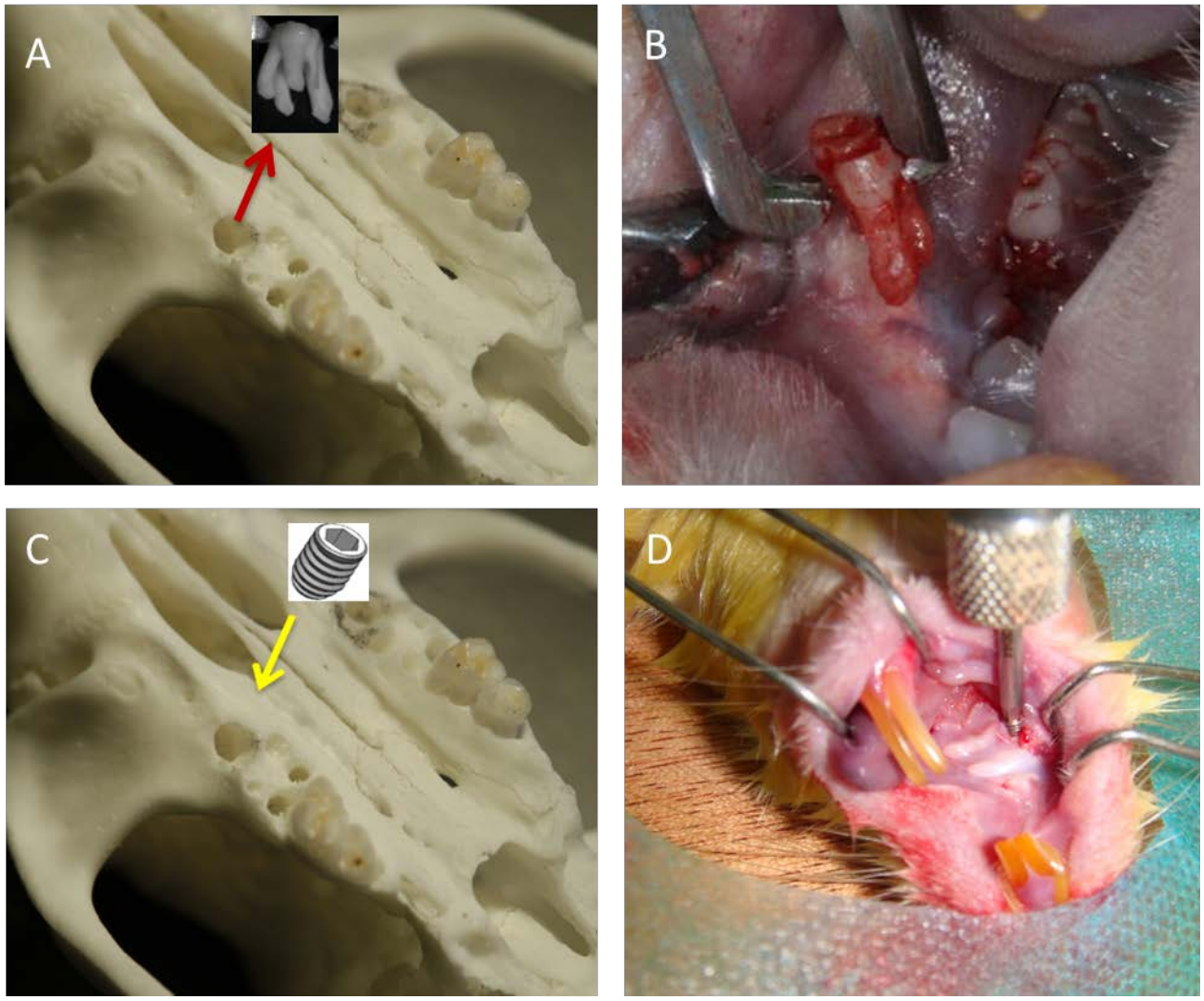

Figure 4.1: Experimental procedure. (A) Diagramatic (yellow arrow) and (B) Actual forceps extraction of maxillary first molar. (C) Arrow indicates diagrammatic and (D) actual unilateral implant placement in mesial root extraction socket of maxillary first molar.

4.4.2 Implant placement

Eighty-four days after ovariectomy surgery, screw-shaped titanium implants with 3 mm length, 2 mm diameter, and 0.22 mm thread pitch (surface-treated implants, Southern Implants, Irene, South Africa) were placed in the extraction sites of the mesial roots of the right maxillary molars (Figure 4.1) and any open wound at the implant site

was closed with sutures. In brief, general anaesthesia was achieved by administration of 2.5% pentobarbital sodium (Chemical Agent Co., Shanghai, China) at 45 mg/kg body weight. The maxillary molars were removed and implant sites were prepared with a dental bur at 1000 revolutions per minute. Subsequently, the implants were placed at the mesial root of each freshly extracted tooth socket until the screw threads were totally concealed in bone under continuous saline irrigation. Ten animals from each group were euthanized for histological assessment either at 28 or 56 days after implant placement (Figure 4.1).

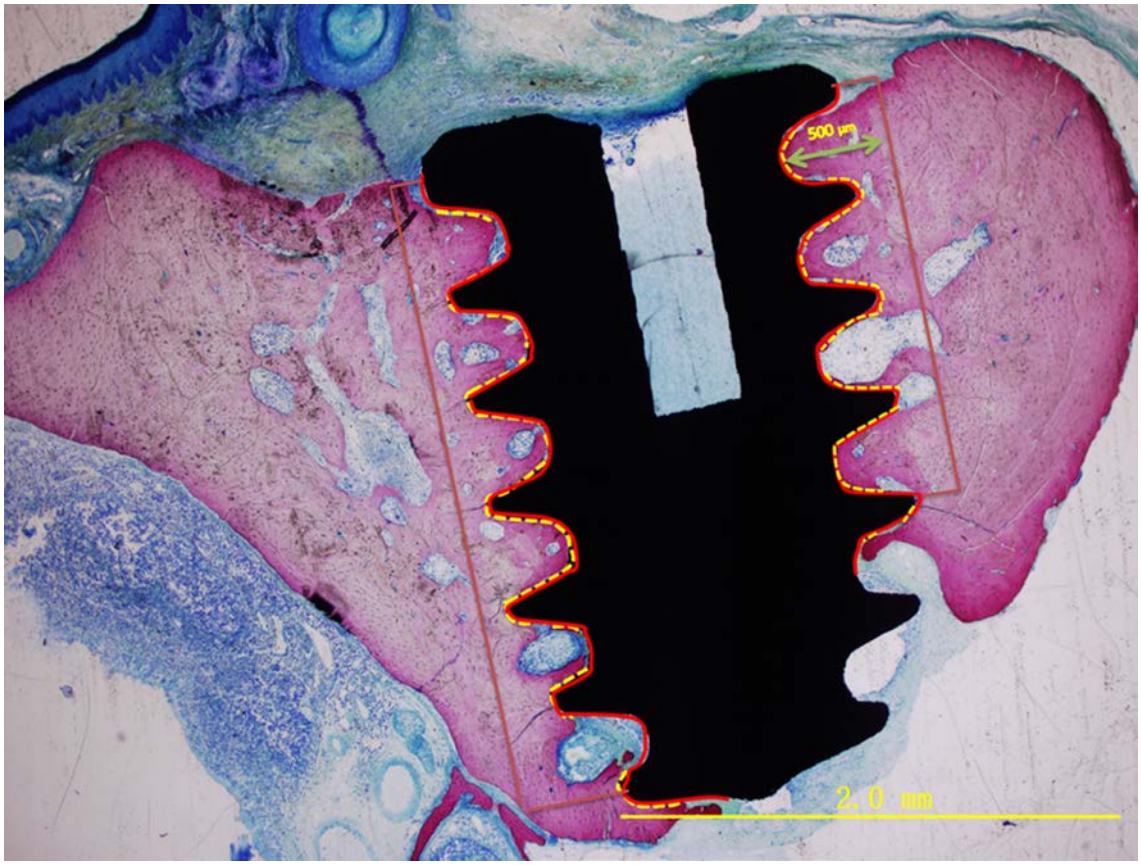

Figure 4.2: Outline of methods used for calculating BIC and BD. Methylene blue-basic fuchsin staining showed bony areas as pink, soft tissue areas as blue, and implant screw appeared as black. BIC areas were coloured with yellow dashes whereas the continuous red lines represented implant surface. Non-contacted areas were interspersed with blue

islands of soft tissue. Two rectangles (with double green arrows) illustrated bilateral 500 µm wide areas used for calculating bone density.

4.4.3 Histological assessment

This section followed the protocol recommended by previous publications.[16] After the animals were sacrificed, a segment of the posterior maxilla surrounding the titanium implants was collected and fixed in 4% neutral formalin for 48 hours. Further procedures were carried out, including trimming, dehydration, permeation, embedding, milling, and staining. Briefly, the specimens were desiccated in a number of graded alcohols and fixed in polyester resin without decalcification. Undecalcified sections of roughly 200 µm were ground to 30-50 µm thick and longitudinal to the implant by an Exakt saw microtome (Exakt, Norderstedt, Germany) and arranged using the previously published method[16]. Two sections were prepared from each implant and stained with methylene blue-basic fuchsin (Sigma-Aldrich, St Louis, MO, USA), and subsequently examined with light microscopy.

4.4.4 Bone formation and osseo-integration analysis

As indicated in the previously published implant research protocols[13, 19], the amount of newly formed bone surrounding the implants was measured using histomorphometric analysis under light microscope. The indices used in this study were: (1) Bone to Implant Contact (BIC), calculated as the proportion of bone directly adhered to implant surface; (2) Bone Density (BD), defined as the percentage of bone in a 500 µm zone adjacent to the implant surface**.** The BIC and BD were calculated using a 500 µm thick area lateral to both sides of the implant surfaces (Figure 4.2). The analyses were done by an experienced examiner to ensure reproducibility of the report.

4.4.5 Statistical methods

Statistical evaluations were carried out using the Sigma Stat statistics package (SPSS Inc., Chicago, IL, USA). Variations in bone quantity within the two groups were evaluated using a t-test and the significance was set as $p<0.05$.

4.5 RESULTS

During the period of post-implantation observation, there was no wound infection, or fatality and no implants were lost.

4.5.1 Validation of rat osteoporosis models at day 84

The OVX model has been previously validated in our studies.[13, 17] In order to confirm the rat osteoporosis (OP) model, DEXA and micro-CT were used to quantify the osteoporosis status at day 84. The DEXA results indicated the bone density from the OVX rats (n=10) was significantly lower than that from the SHAM rats (n=10) (Figure 4.3). Micro CT of the rat's maxilla showed more porous bone in the OVX group than in the SHAM counterpart (data not shown). These results confirmed the successful induction of osteoporotic-like conditions in this model.

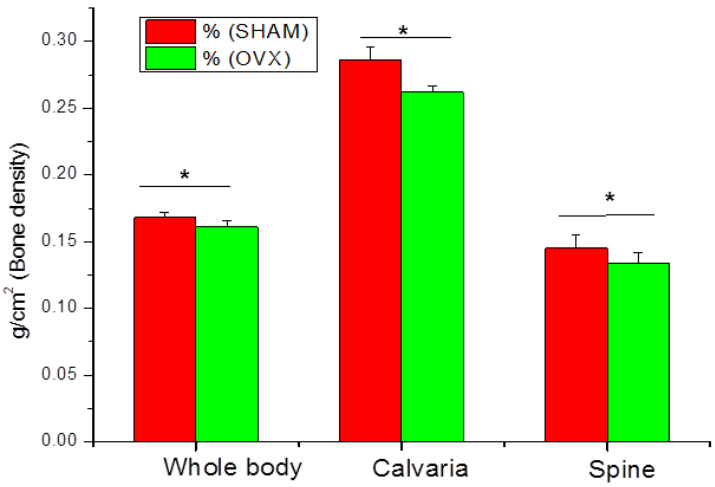

Figure 4.3: DEXA measurement. Graph showed confirmation of osteoporosis model of OVX.

4.5.2 Histological analysis

The method used for the histomorphometric quantitative comparison of osseo-integration in the two treatment groups has been previously presented.[13] The results of this study illustrate descriptive analyses of the histological characteristics that are pertinent to osseo-integration. Figures 4.4, 4.5 and 4.6 present the histological features of the bone healing 28 days (4 weeks) and 56 days (8 weeks) after implant insertion in the two groups. At 4 weeks and 8 weeks, the OVX group (n=10) had significantly ($p<0.05$) less BIC and BD than the SHAM group (n=10) (Figure 4.4). In terms of BIC, there was a slight increase in 8 weeks compared with 4 weeks in both groups; however,

no significant improvement was detected in BD between 4 and 8 weeks for either group (Figure 4.4).

At 28 days, in the OVX group (Figure 4.5 C&D), there appeared to be less recently produced bone associated with the implants compared with the SHAM groups (Figures 5 A&B). Additionally, the cancellous bone appeared to have less mineralized trabeculation present in the OVX group than in the SHAM group. In contrast with the OVX group, the morphology of the recently formed bone neighbouring the implants in the SHAM group displayed more bone surrounding the implants in terms of the matrix width and the unbroken link of mineralized mass surrounding the implant surface.
Furthermore, as compared with the SHAM group (Figure 4.7A), the OVX group (Figure 4.7B) had fewer osteoblasts in the freshly produced bone matrix (darkly stained) neighbouring the implant and the bone matrix near the implants was slender and sporadic. The presence of osteoclastic activity was commonly observed in the recently produced bone in the OVX group (Figure 4.7B).

At 56 days after implant placement, the histological data exhibited more bone to implant contact than at 28 days in both groups (Figures 4.6). The differences in terms of BD in the SHAM group were minimal between days 28 and 56, as the recently formed bone on the implant surface became more mature (palely stained) with time rather than increasing in volume (Figure 4.5&4.6). In the OVX group, the quantity of newly formed bony tissue surrounding the implant surface (Figure 4.6 C&D) was poorer than the new bone surrounding the implants in the SHAM group (Figures 6 A & B). Greater BIC and BD were more obvious in the middle sections than in the coronal aspects. The cancellous bone distant from the implant surface exhibited fewer mineralized trabeculae

in the OVX group than in the SHAM group. In contrast to the SHAM group (Figure 4.7C), the OVX group (Figure 4.7D) showed more signs of both osteoblastic and osteoclastic activity and bone turnover in the bone adjacent to the implants. The osteoclastic activity was very obvious in the OVX group (Figure 7D) at high magnification.

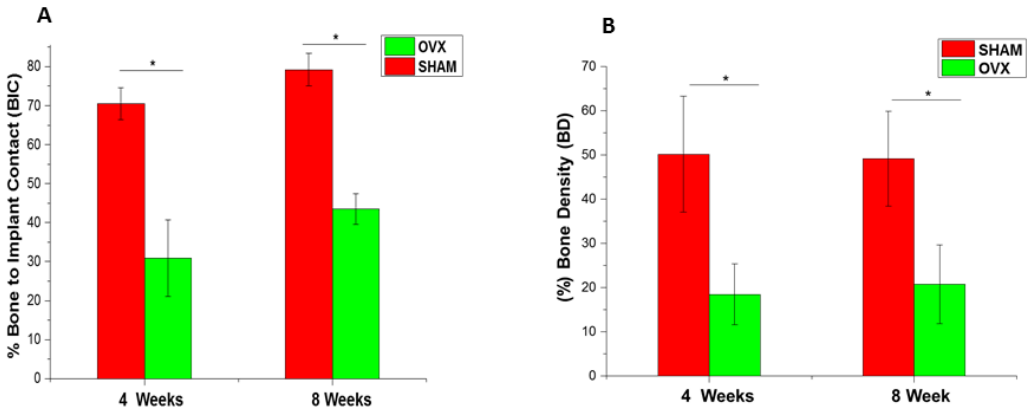

Figure 4.4: (A) Graphs of bone-implant contact (BIC) at 4 weeks and 8 weeks. BIC in SHAM groups was significantly higher than that in the OVX. (B) Graphs of bone density (BD) at 4 weeks and 8 weeks. BD in SHAM group was better and statistically more significant than in the OVX group.

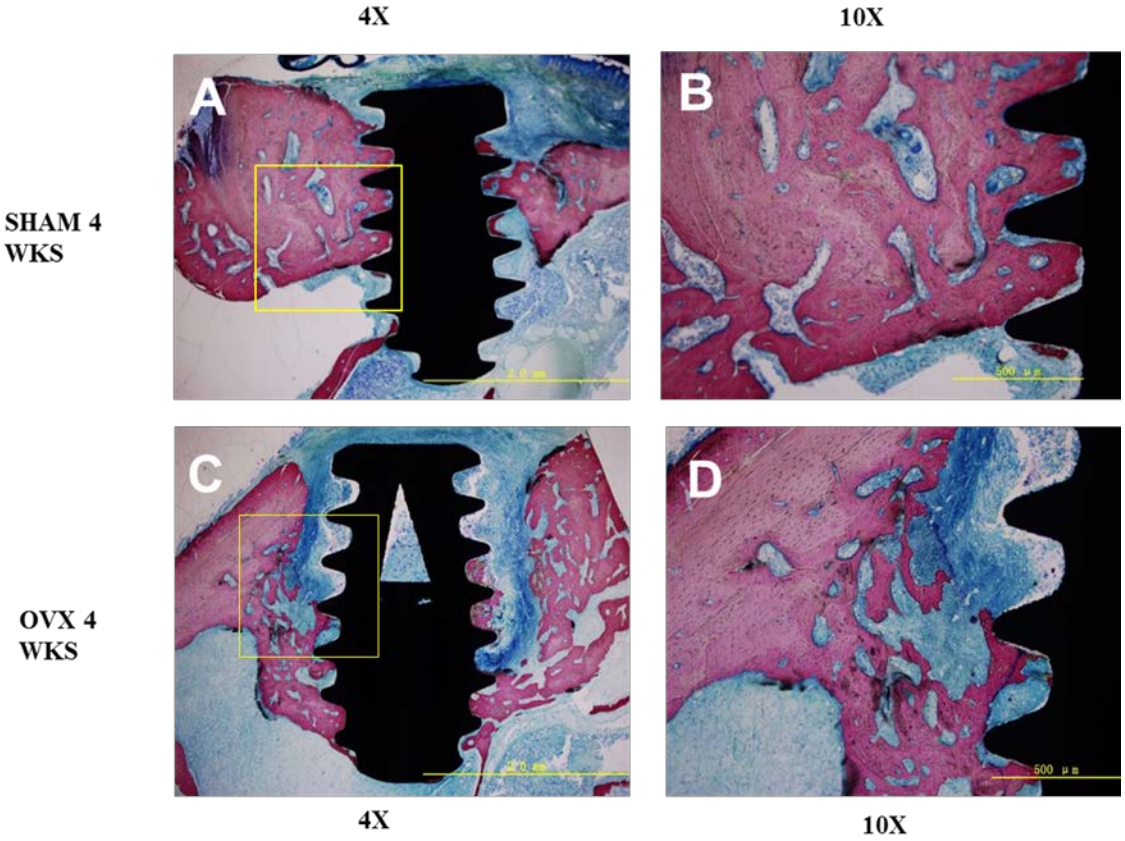

Figure 4.5: Longitudinal section of the implants stained with methylene blue-basic fuchsin at 4 weeks. At 4 weeks the bone tissue of the SHAM was more compact than the OVX, and osseo-integration in the SHAM was better than in the OVX. Bone to implant contact (BIC) and bone density (BD) were lower in OVX (C and D) than in the SHAM (A and B) at both low magnification (A and C), and higher magnification (B and D). Bar=2 mm for 4x magnification and bar=500 µm for the 10x magnification. (SHAM (A) and OVX (C) at 4x magnification, SHAM (B) and OVX (D) at 10x magnification).

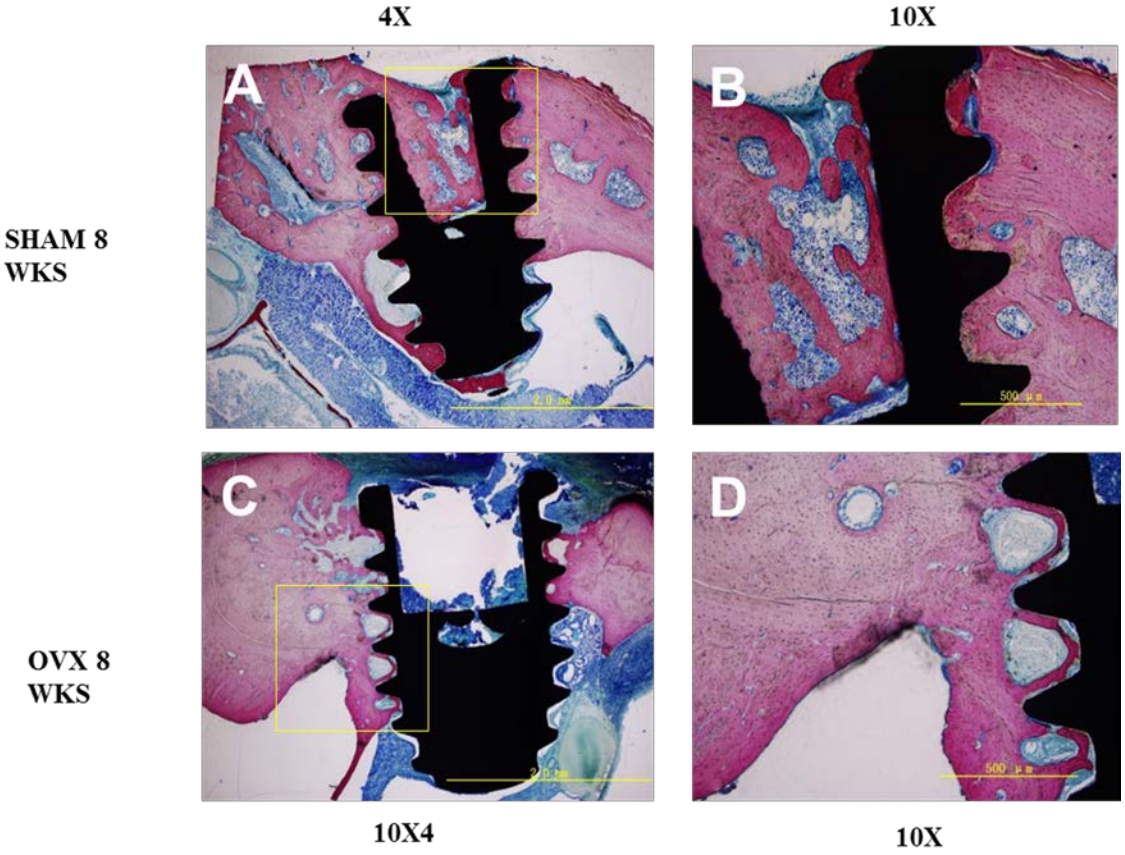

Figure 4.6: Longitudinal section of the implants stained with methylene blue-basic fuchsin at 8 weeks. Bone to implant contact (BIC) and bone density (BD) were lower in OVX (C and D) than in SHAM group (A and B) at both low magnification (A and C), and higher magnification (B and D. Bar=2 mm for 4x magnification, and bar=500 µm for the 10x magnification. (SHAM (A) and OVX (C) at 4x magnification, SHAM (B) and OVX (D) at 10x magnification).

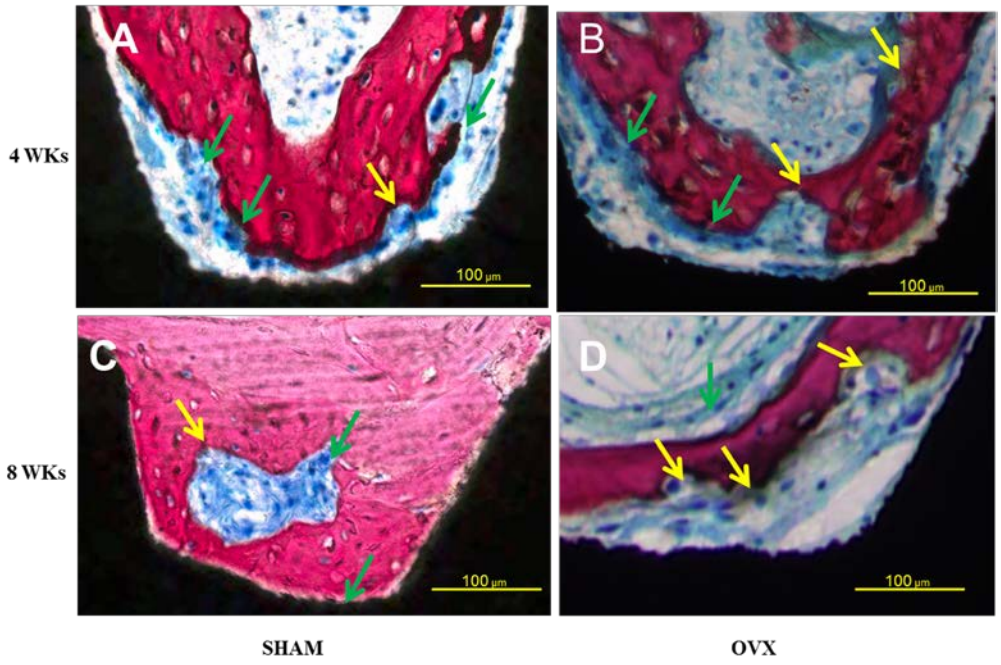

Figure 4.7: Osteoclasts and osteoblasts in cancellous areas in SHAM and OVX groups at 28 and 56 days. The yellow arrows (A, B, C and D) indicate the osteoclasts and the green arrows (A, B, C and D) show the osteoblasts. More osteoblastic activity was found in the SHAM group at 4 weeks (A) and 8 weeks (C) whereas in the OVX group, osteoclastic activity was more obvious at both 4 weeks (B) and 8 weeks (D).

4.6 DISCUSSION

There is evidence to suggest that clinical implant success rates are diminished with certain systemic conditions, such as osteoporosis [3, 19, 20]. Indeed, decreased bone quality and quantity, such as that encountered in osteoporosis, may have an adverse effect on osseo-integration[13]. Despite the proposition of undesirable consequences of low bone quantity or osteoporosis on the success rate of implant therapy, both animal and human studies have been able to prove that immediate titanium implantation is not an absolute contraindication in poor quality bone subjects [3, 16, 21]. However, the early bone healing process around dental implant is not fully understood, especially in the lower bone quality region of the posterior maxilla in the OVX rat model.

Owing to its unique anatomy, the posterior maxilla presents several challenges, such as poor bone quality and the presence of the maxillary sinus. These challenges may be overcome with training and experience. Nevertheless, the challenges presented by both local and systemic compromised clinical scenarios require suitable animal models in order to study implant performance under these conditions. The current study has demonstrated that immediate implantation following extraction in the model established in this study is a predictable way, with minimal complications, to study implant placement, as no implant loss was observed during the period of study. Although the model used in this study can be considered technique sensitive, with appropriate experience, immediate implant surgery may be considered a predictable technique for implant placement in the posterior maxilla in the rat model.

In the present study, it was observed that after 28 days post-implant placement, the OVX group had reduced osseo-integration, as evidenced by lower bone to implant contact (BIC) and bone density (BD) compared with the SHAM group. This finding may signify that the OVX group had a greater amount of bone resorption as illustrated by prominent osteoclastic activity in the newly formed bone around the implants [22]. As a result, the BIC and BD in the SHAM group were superior to those in the OVX group at 28 days. This result confirms that the osteoporosis induced in the OVX rats is a good model for investigating the potential cellular and molecular mechanisms of osseo-integration, as well as the development of potential interventions, in osetoporosis subjects.

Notably, at 56 days, BIC was significantly improved compared with that at 28 days. Interestingly, BIC and BD in the SHAM group were still higher than in the OVX group. These outcomes suggest that there was continuing osteoblastic activity on the implant surfaces over the duration of the study. This is consistent with recent evidence showing

that titanium implant surfaces could stimulate the expression of TGF-β/BMP and non-canonical WNT/Ca2+ signaling genes, inducing the osteogenic differentiation of mesenchymal stem cells.[23] In other words, implant surfaces act as excellent osteoconductors and osteoinductors for the bone healing process. However, the overall result for the OVX group is still inferior to that of the SHAM group, suggesting altered osteogenic properties in OVX subjects. Indeed, it was noted that in OVX rats more signs of both osteoblastic and osteoclastic activity and higher bone turnover were found in the bone adjacent to the implants. Furthermore, the osteoclastic activity was more in evidence in the OVX than in the SHAM controls, indicating that further intervention should be sought to enhance osseo-integration in osteoporotic subjects.

4.7 CONCLUSION

This study shows that osseo-integration in osteoporotic rats in the posterior maxilla is significantly inferior to that in the SHAM control, especially in the early healing period, indicating that lower local bone quality in OVX rats can reduce early bone formation on implant surfaces.

4.8 ACKNOWLEDGEMENTS

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Chapter 5: The Effects of Simvastatin on Osseo-integration around Titanium Implants in Posterior Maxilla of Osteoporotic Rats (Part II)

In the process of submission to the Journal of Clinical Oral Implants Research

The Effects of Simvastatin on Osseointegration around Titanium Implants in Posterior Maxilla of Osteoporotic Rats
(Part II)

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Statement of Contribution of Co-Authors for Thesis by Published Paper:

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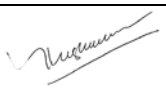
Doan N Signature  Date: 5 February 2014	• Concept and design, • Analysis and interpretation of research data; • Drafting significant parts of a work, • Critically revising it so as to contribute to the interpretation.
Du Z	• Concept and design, • Analysis and interpretation of research data; • Critically revising it so as to contribute to the interpretation
Xiao Y	• Concept and design, • Analysis and interpretation of research data; • Drafting significant parts of a work, • Critically revising it so as to contribute to the interpretation.
Reher P	• Analysis and interpretation of research data; • Critically revising it so as to contribute to the interpretation.
Crawford R	• Critically revising it so as to contribute to the interpretation.
Ivanovski S	• Critically revising it so as to contribute to the interpretation.
Xiao J	• Analysis and interpretation of research data;
Yan F	• Concept and design, • Analysis and interpretation of research data;

Chen J	• Concept and design,
Xia W	• Concept and design, • Analysis and interpretation of research data;

Principal Supervisor Confirmation

I have sighted email or other correspondence from all Co-authors confirming their certifying authorship

Professor Yin Xiao		5 February 2014
Name	Signature	Date

5.1 ABSTRACT

Objective: This study aims to evaluate the relationship between implant placement, poor quality bone, simvastatin, and osseo-integration of surface-treated implants in the posterior maxilla of osteoporotic rats.

Materials and methods: Sixty-four female Sprague-Dawley rats, aged 3 months old were used in this study, divided into three groups: Sham-operated (SHAM; n=20), ovariectomized (OVX; n=20) and ovariectomized treated with simvastatin (OVX+SIM; n=20). Two rats from the SHAM and two from the OVX groups were used to verify osteoporosis. Eighty-four days following ovariectomy, screw-shaped titanium implants were immediately placed into mesial root sockets of the posterior maxilla. Simvastatin was administered orally at 5 mg/kg each day after the implant placement in the OVX+SIM group. The animals were sacrificed at either 28 or 56 days from the date of implant insert and the undecalcified tissue sections were processed for histological analysis. The osseo-integration indices used were: bone formation rate (BFR), bone to implant contact (BIC), and bone density (BD).

Results: The osseo-integration indices (BFR, BIC and BD) in the three groups demonstrated significant differences among the SHAM > OVX+SIM > OVX group, which implied that simvastatin could promote bone mineralization in OVX rats.

Conclusion: This study shows for the first time that simvastatin can positively affect the osseo-integration indices, and successfully promoted osseo-integration in the posterior maxilla in OP rats.

5.2 KEYWORDS

- Simvastatin
- Dental implants
- Implant success
- Implant survival
- Osteoporosis
- Outcomes
- Posterior maxilla
- Rats

5.3 INTRODUCTION

The phenomenal pace of dental implant development in the last two decades has led to widespread studies in both humans[1] and animals [2]. As the implant success rate improves, dental implantologists have to deal with much more complex issues encountered in those patients with medical conditions such as osteoporosis.[3] Osseo-integration or the process of incorporation of a dental implant into the beneficiary bone, consists of a series of incidents that can be affected by several issues such as site selection, surgical techniques, systemic and local conditions, and medication used.[2-4] There is sufficient evidence that success rates of implant clinical procedures markedly reduce with age and certain systemic conditions, such as osteoporosis.[4-6] Poor bone quality and quantity, such as those found in osteoporosis, may produce an unfavourable effect on osseo-integration[2,3]. In spite of the proposition of the undesirable consequence of dwindling bone quantity or osteoporosis on the success rate of implant therapy, animal research has been able to demonstrate the enhanced properties of statins on osseo-integration in poor quality bone osteoporotic rats.[7]

In humans, poor quality bone is commonly found in post-menopause women.[5, 8].In rats osteoporosis can be induced using a model through ovariectomy as suggested by Du et al. (2009).[2] However, most such animal studies have not used the surface-treated implants (STIs) that have been gaining popularity recently. Osteoporosis is a medical condition that has been described as having undesirable consequences on bone formation in the course of dental implant osseo-integration, and as such, it is regarded as conducive to implant failure.[9-13] Several remedial methods have been recommended for the enhancement of osseo-integration of dental implants in osteoporotic patients. [2, 14-16] Presently, the majority of medications employed in treatment of osteoporosis control bone resorption by reducing bone turnover and consequently decreasing bone loss.[2] Theoretically, medications that could improve bone formation would be most likely to increase osseo-integration of dental implants in cases of poor bone condition, for instance that found in osteoporotic patients.

One of the common statin derivatives is simvastatin. It is a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor that is commonly used as a cholesterol-lowering drug and impedes hepatic cholesterol biosynthesis.[2, 17, 18] Many

animal and human studies have been carried out to clarify the clinical significance of statins. Recent and existing publications have indicated a positive effect of statins on bone mineral density (BMD).[17,19,20] The majority of these experimental and epidemiological studies have revealed that statins exert beneficial effects on bone metabolism,[18-23]and fracture risk[24,25]. The most probable explanation for this is that numerous statin medications, including simvastatin, enhance the mRNA manifestation of bone morphogenetic protein (BMP-2) in osteoblasts, with a consequent surge in bone growth when inoculated subcutaneously next to the murine calvaria.[19] Hence, statins have been suggested as prospective agents in the management of osteoporosis. Earlier findings also propose that simvastatin can stimulate osteogenesis around smooth surface titanium implants.[26-28]

Similarly, rough surface treated titanium implants such as those found in Straumann® SLA implants and equivalents, were found to be osseo-conductive to bone forming cells (osteoblasts) through up-regulating mRNA expression of bone morphogenetic protein (BMP-2).[29] Previous studies also suggested that STIs can stimulate osteogenesis around titanium implants[26-28, 30] by up-regulating the expression of TGF-β/BMP and non-canonical WNT/Ca2+ signaling genes.[30]

In a previous study [2], it has been stated that simvastatin enhances the osseo-integration of machine surface titanium in the tibias of osteoporotic rats. However, the mechanism by which simvastatin affects osseo-integration of ovariectomized (OVX) rats is not specified.

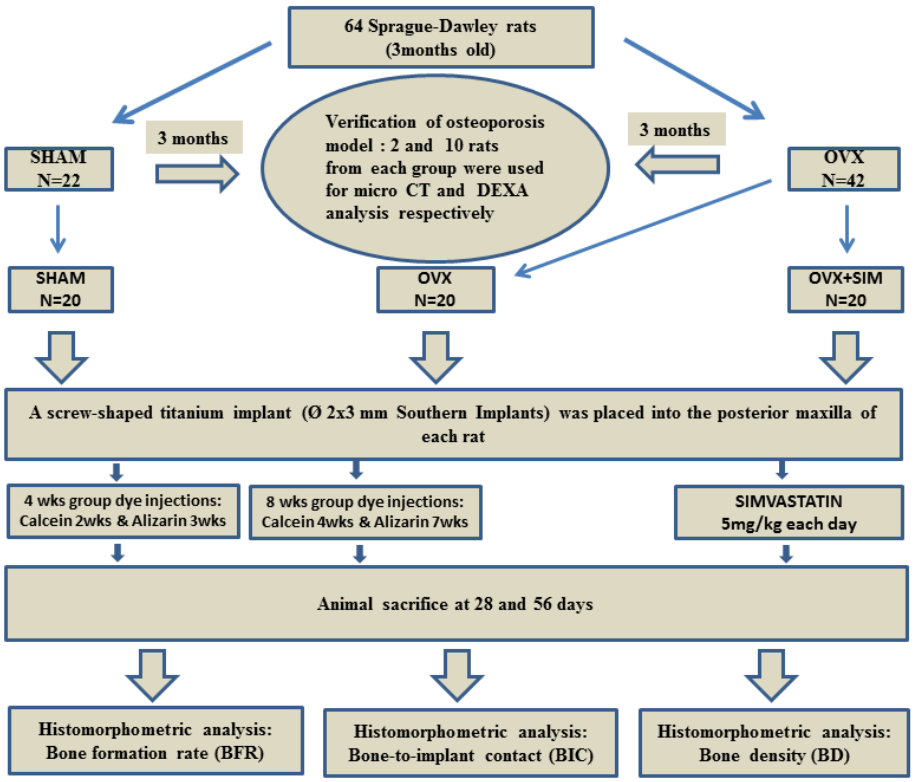
There have been many published studies looking at the effects of simvastatin on osseo-integration of dental implants in osteoporotic subjects; however, not one of them has concentrated on STIs in the posterior maxilla of osteoporotic rats. This study attempts to be the first of its kind to fill this knowledge gap. The working hypotheses are: (1) Osteporotic (OVX) rats have lower osseo-integration indices than normal (SHAM) rats; (2) Simvastatin in conjunction with surface-treated implants (STIs) can enhance osseo-integration in osteoporotic rats. Thus, the aim of this study is to evaluate these hypotheses by assessing the association between bone formation during osseo-integration of surface-treated implants in the posterior maxilla of osteoporotic (OVX) rats treated with simvastatin.

5.4 MATERIALS AND METHODS

5.4.1 Experimental Design

This study was conducted following a protocol approved by the Animal Care and Use Committee of Fujian Medical University, and a similar research approach employed previously by Du et al. (2009) [2, 6] and is described in Figure 5.1. In brief, sixty-four 3-month-old female Sprague-Dawley rats (SLAC Laboratory Animal Co. Ltd, Shanghai, China), were segregated initially into two groups: initial SHAM (n= 22) and initial OVX (n= 42). A total of 4 rats, 2 from each group, were sacrificed for histomorphometric analysis. A further 10 rats from each group were used for DEXA analysis to verify the osteoporosis status of the animals. By using a randomized group plan, the 3 study groups were labelled as sham-operated (SHAM, n= 20), ovariectomized (OVX, n= 20) and ovariectomized with simvastatin therapy (OVX+SIM, n= 20). For the OVX and OVX+SIM groups, both parts of the ovaries were uncovered, and totally detached by an abdominal dissection technique. For the SHAM group, the ovaries were opened up and a similar amount of fat adjoining each individual ovary was detached. Successively, the fascia and skin were closed and sutured.[2] Industrial laboratory rat food (Experimental Animal Centre of Zhejiang University, China) and water were available *ad libitum*. Osteoporotic condition as a consequence of ovariectomy was checked by slaying two rats from each group and gathering the proximal tibial metaphyses and uterine horns 56 days after ovariectomy as detailed earlier [2] Simvastatin was dispensed orally at 5 mg/kg every day following the insertion of the implant in the OVX+SIM group.

Figure 5.1 Experimental procedure flow chart



5.4.2 Implant placement

Twenty-eight and 56 days following ovariectomy surgery, endosseous screw-shaped titanium implants (length=3 mm, diameter=2 mm, thread pitch =0.22 mm, Southern Implants, Irene, South Africa) were placed in the freshly extracted mesial molar root and any open wound at the implant site was closed with sutures. In brief, general anaesthesia was achieved by dispensation of 2.5% pentobarbital sodium (Chemical Agent Co., Shanghai, China) at 45 mg/kg body weight. The maxillary molars were removed and implant sites were drilled with a dental bur at 1000 revolution per minute. Specially designed surface-treated implants, measuring 3 mm in length, with a 2 mm diameter, and thread pitch of 0.22 mm, were used in this study (Southern Implants, Irene, South Africa). The implants were placed at the mesial root socket of each freshly extracted tooth till the screw threads were entirely submerged in bone under continuous saline irrigation, and finally the soft tissue was closed and sutured. Simvastatin was given orally at 5 mg/kg per day following implant placement to the OVX+SIM group. Saline was provided as a placebo to the remaining two groups. Ten

animals from every group were slaughtered either at 28 days (4 weeks) or at 56 days (8 weeks) following implant placement.

5.4.3 Fluorescent bone markers

Staining procedure: for the 4-week groups, calcein green and alizarin red were used at 14 days and 21days after surgery respectively using transabdominal injection, and for the 8-week groups, calcein (10 mg/kg) and alizarin red (30 mg/kg) were administered at 28 days and 49 days respectively post-surgically. The staining of newly formed bone at both 28 and 56 days is shown in figure 2. A Laser Confocal Microscope (with CLSM lens) was used to acquire and compile the images for calculation of bone mineralization/formation rate, then the IPP 6.0 software was employed for further analysis. These quantities were correlated to the histomorphometric study of osseo-integration indices of bone formation as shown in figure 2. Bone formation rate, in this context, is described as the band width (µm) of the fluorescence stained bone formed in a defined time interval (days).

5.4.4 Histological assessment

After the animals were sacrificed, a segment of the posterior maxilla surrounding the titanium implants was collected and fixed in 4% neutral formalin for 48 hours. Further procedures were carried out including: trimming, dehydration, permeation, embedding, milling, and staining, as follows. The specimens were desiccated in a number of graded alcohols, and fixed in Technovit 7200 acrylic resin devoid of decalcification. Undecalcified sections roughly 30-50 µm thick and longitudinal to the implant, were excised with an Exakt saw microtome (Exakt, Norderstedt, Germany) and arranged using the bone grinding slice method[2]. The 30-50µm thick slides were immersed in methylene blue solution at 60ºC for 15 minutes then filter paper was used to blot the excess solution thoroughly. The slides were then transferred into basic fuchsin solution at room temperature for 5 minutes, blotting paper was again used to absorb water to achieve total dryness. 95% and 100% ethanol was employed for dehydration; xylene was used to clear; and finally a small drop of resin was carefully added to the coverslip to seal the slide.

Three sections were prepared from each implant and stained with methylene blue-basic fuchsin (Sigma-Aldrich, St Louis, MO, USA), and subsequently examined with light microscopy. The osseo-integration indices used were: (1) bone formation rate

(BFR), expressed as the band width (µm) of bone formed in a defined time interval (days); (2) bone to implant contact (BIC), defined as the percentage of bone in direct contact with implant surface; and (3) bone density (BD), calculated as the percentage of bone in a 500 µm area laterally to the implant surface.

5.4.5 Statistical methods

Variations in bone quantity among the three groups were measured by one-way analysis of variance (ANOVA) followed by Fisher's LSD post hoc test (a=0.05). Statistical evaluations were carried out with the Sigma Stat statistics package (SPSS Inc., Chicago, IL, USA).

5.5 RESULTS

During the post-implantation observation period, there was no wound infection, no fatality, and no implants were lost.

5.5.1 Histological analysis

The presentation of the results of this study is similar to that found in our preceding paper part (I) and the method of histomorphometric quantitative comparison of osseo-integration in the 3 treatment groups published earlier[2]. Figure 5.2 shows the histological features of the bone development 28 and 56 days after implant insertion in the 3 groups.

5.5.2 Validation of rat osteoporosis models at 56 days (8 weeks)

The confirmation of the rat OP model was done as in part I of the previous paper.

5.5.3 Bone formation rate around dental implants using fluorescence staining at day 28 (4 weeks) and day 56 (8 weeks)

The corresponding fluorescence marked bone neighbouring an investigated implant with calcein emerging as green and alizarin displaying as red. The thread and peak distance is 0.22 mm. Under laser confocal compound image of fluorescent staining, the studied implant appeared as black in the background (Figure 5.2).

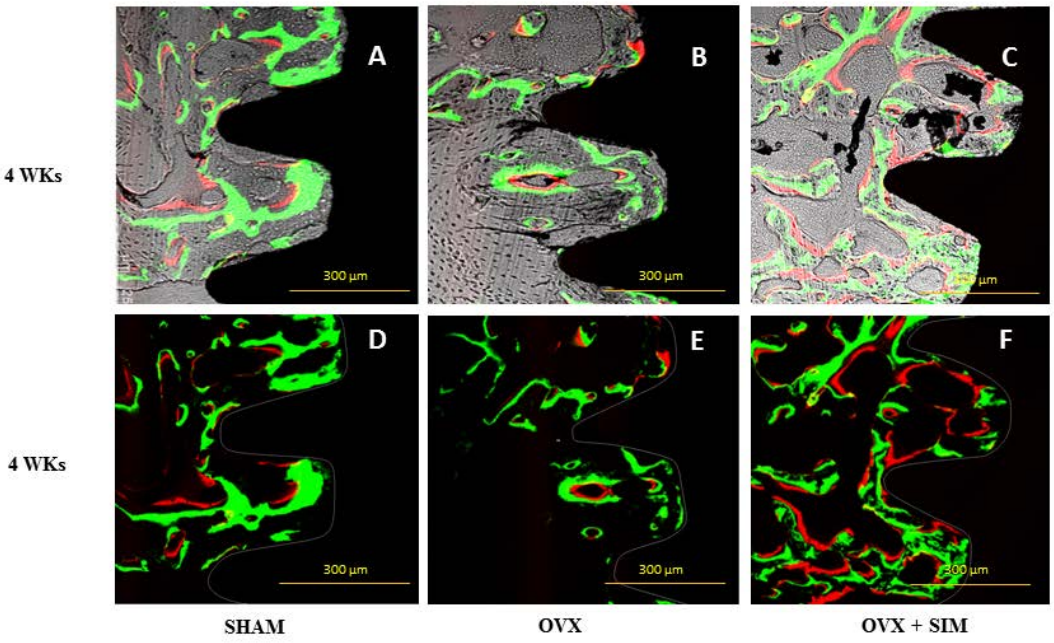


Figure 5.2: Bone formation using fluorescence staining under laser scanning confocal microscope after 28 days (4 weeks). A, B, and C show the compound image of fluorescent staining with visible implant in the background. D, E, and F illustrate compound images of fluorescent staining with implant removed from the background. The corresponding fluorescence-labelled bone surrounding a studied implant shows calcein as green and alizarin as red. The order of staining intensity of mineralized bone by the three groups was found to be: SHAM > OVX+ SIM >OVX. With addition of simvastatin not only was more mineralized bone formed but it emerged to become closer to the implant surface. The distance between thread and peak was 0.22 mm.

The corresponding fluorescence-labelled active bone formation surrounding a studied implant showed calcein as green and alizarin as red. The qualitative observations of the sections using a fluorescence microscope showed that there was constant bone activity in the neighbourhood of the implants throughout the healing period, as great quantities of calcein, and smaller amounts of alizarin were identified in

this zone. A strong labelling of the fluorochromes disclosed uneven outlines in the vicinity of the implants and in the surrounding and adjacent areas. Bone formation, bone resorption, and bone remodelling voids were detected around the control (SHAM), OVX and the test (OVX+SIM) implants (Figure 5.2). In the SHAM group, the calcein staining (1st label) was found very significantly, while the contrary, less alizarin (2nd label) could be seen near the (OVX) at 4 weeks (Figure 2 B) and 8 weeks (Figure 5.3 E) as compared with the SHAM (Figure 2 A and D) and OVX+ SIM (Figures 2 C and F). The order of visible quantity of alizarin was: SHAM> OVX+SIM > OVX (Figures 2 and 3). The existence of fluorochromes could be detected adjacent to the implants in all three groups, though this occurrence appeared to be most frequent adjacent to the control (SHAM) implants. The latter inspection showed strongly-stained calcein borders in the SHAM implant boundaries, mainly in the threads situated in the marrow space section. The order of staining intensity of mineralized bone by the three groups was found to be: SHAM > OVX+ SIM >OVX. In the OVX group, the stained mineralized bone was further away from the implant surface compared with the SHAM and OVX+SIM groups. With addition of simvastatin not only was more mineralized bone formed but it emerged to become closer to the implant surface (Figures 5.2 C & F and figure 5.3 C & F).

At both 4 weeks (Figure 5.2) and 8 weeks (Figure 5.3), there appears to be less newly formed bone in the vicinity of the implant in the OVX (Figures 5.2 and 5.3: B & E) group than in the SHAM group (Figures 5.2 and 5.3: A & D) and OVX+SIM (Figures 5.2 and 5.3: C & F) groups.

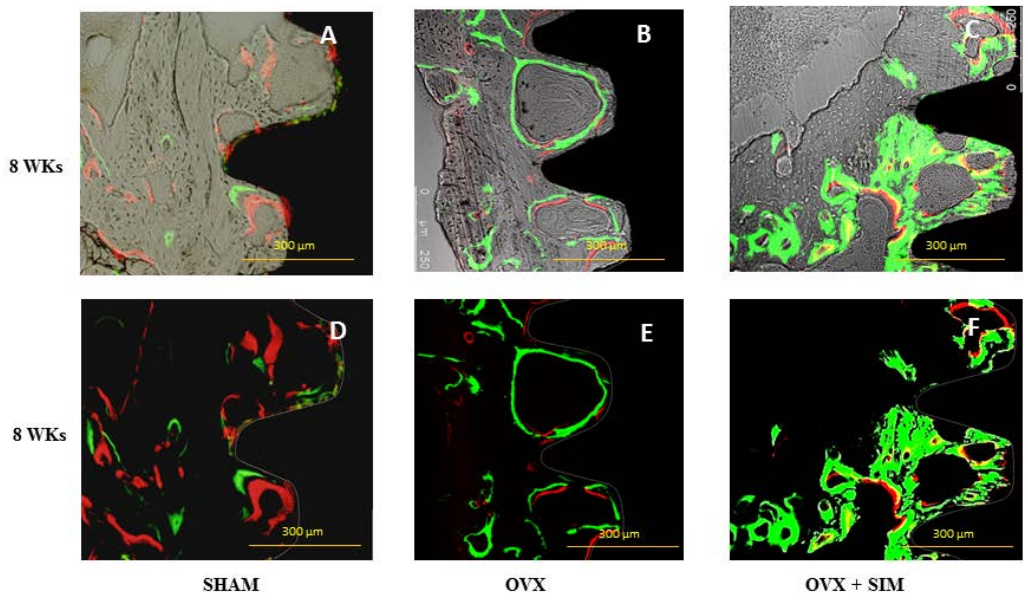


Figure 5.3: Bone formation using fluorescence staining under laser scanning confocal microscope after 56 days (8 weeks). A, B, and C show a compound image of fluorescent staining with implant visible in the background. D, E, and F illustrate compound images of fluorescent staining with implant removed from the background. The corresponding fluorescence-labelled bone surrounding a studied implant illustrates that the gaps between calcein (as green) and alizarin (as red) were smaller, indicating a slower bone formation rate (BFR), with the best BFR found in the SHAM and the least in OVX. Inclusion of simvastatin helped to improve bone BFR but also made mineralized bone appear closer to the implant surfaces. The distance between the thread and peak was 0.22 mm.

Bone formation rate of the OVX group (Figure 5.4) is significantly less than that of the SHAM and OVX+SIM groups at both 4 ($p<0.01$) and 8 weeks ($p<0.005$). BFR has slowed down in the three groups at 8 weeks. Also at 8 weeks, the OVX+SIM group has experienced the least reduction in BFR. The order of BFR was SHAM>OVX+SIM>OVX.

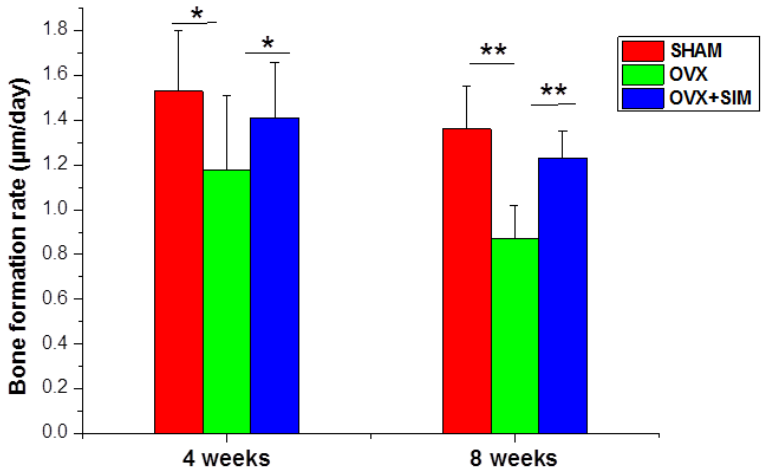


Figure 5.4: Graph illustrating bone formation rate using fluorescence staining after 4 weeks and 8 weeks. The differences in BFR between OVX and SHAM and OVX and OVX+SIM were statistically significant at 4 weeks (*$p<0.05$) and 8 weeks (**$p<0.005$) respectively. BFR has slowed down in the three groups at 8 weeks. Also at 8 weeks, the OVX+SIM group experienced the least reduction in BFR. The order of BFR was SHAM>OVX+SIM>OVX.

5.5.4 Results of osseo-integration as measured by bone indices (BIC, and BD) at 4 weeks (28 days) and 8 weeks (56 days) using methylene blue-basic fuchsin staining

The results of methylene blue-basic fuchsin staining at 4 weeks and 8 weeks are shown in Figure 5.5. At 4 weeks and 8 weeks, the OVX group had significantly (*$p<0.05$) lower BIC (Figure 5.6 A) and BD (Figure 5.6 B) than both the SHAM and OVX+SIM groups. However, in the SHAM group, the bone density seemed to slow down at 8 weeks but there was no statistical significance between 4 weeks and 8 weeks (Figures 5.5 D & A).

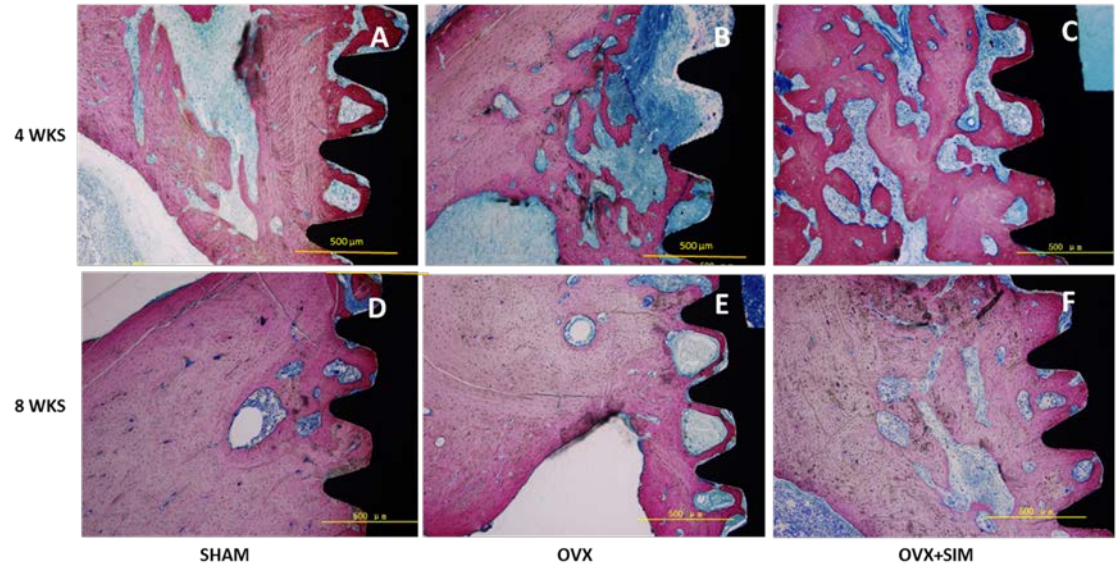


Figure 5.5: Histological evaluation of bone to implant contact (BIC) and bone density (BD) at 4 weeks (A, B, and C) and 8 weeks (D, E, and F) (4x magnification using methylene blue-basic fuchsin staining). At 4 weeks: (A) SHAM, (B) OVX, and (C) OVX+SIM. At 8 weeks: (D) SHAM, (E) OVX, and (F) OVX+SIM. Compared with 4 weeks, there appears to be more new bone formation at 8 weeks near the implant in the three groups and the order is SHAM>OVX+SIM>OVX.

5.5.5 Day 28

In the OVX group (Figure 5.5 B), there was less freshly developed bone near the implant in contrast to the SHAM (Figure 5.5 A) and the OVX+SIM (Figure 5.5 C) groups at day 28 after implant placement. There was a smaller number of osteoblasts in the recently established bone bed near the implant, and the bone matrix around the implant was slim and sporadic (Figure 5.5 B). The presence of osteoclastic activity was seen in the freshly established bone in the OVX group (Figure 5.5 B). Additionally, the cancellous bone further from the implant exterior appears to have fewer mineralized trabeculae in the OVX group than in the SHAM and OVX+SIM groups (Figures 5.5 A-C). At day 28, the morphology of the newly produced bone near the implants in both the SHAM and OVX+SIM groups displayed similar features (Figures 5.5A & C). In contrast to the OVX group, both the OVX+SIM and SHAM groups displayed more bone surrounding the implants in terms of the matrix width and the continuous link of mineralized mass surrounding the implant surface (Figures 5.5 A-C). In the OVX+SIM

group, the majority of the newly formed bone matrix surrounding the implant seemed to be not as mature as in the SHAM group (Figs 5.5 A and C).

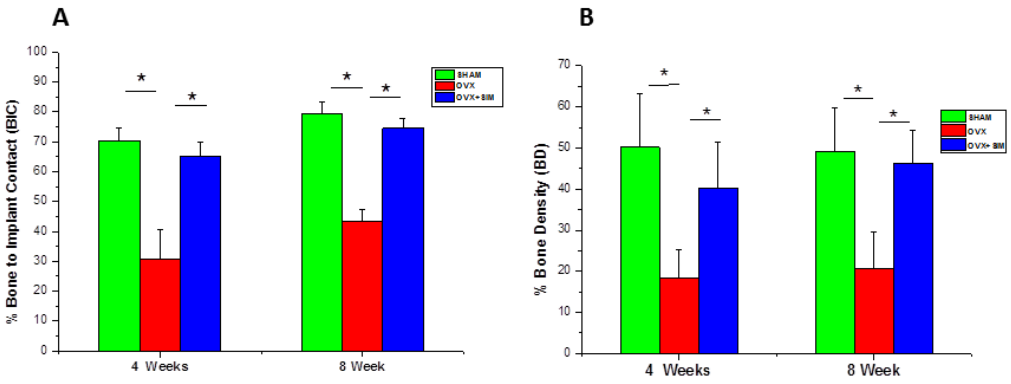


Figure 5.6: Graphs of bone to implant contact (BIC) and bone density (BD). BIC (A) and BD (B) illustrate the inferiority and statistically significant differences of the OVX groups as compared with the SHAM ($P<0.05$) and OVX+SIM groups ($P<0.05$). Compared with the 4 week groups, the 8 week groups show BIC increased by the three groups and BD appears to be denser in the OVX and OVX+SIM groups but not in the SHAM group.

5.5.6 Day 56

At 56 days (Figures 5.5 D-F and 5.6 D-F) after implant placement, the histological data disclosed more newly created bone concealing the implant surface than at 28 days in all 3 groups (Figures 5.5.5A-C vs. Figs 5 D-F). The differences in both the SHAM and OVX+SIM groups were minimal, as the recently created bone on the implant surface turned out to be denser with time (Figures 5.5A &C vs. Figures 5.5 D &F). In the OVX group (Figure 5.5 E), the quantity of new bony tissue surrounding the implant surface was less compared with the new bone surrounding the implant shown in

the OVX+SIM and SHAM groups (Figures 5.5 D & F). In contrast to the SHAM and OVX+SIM groups, the OVX group shows more signs of both osteoblastic and osteoclastic action in the bone base adjacent to the implants (Figures. 5.5 B and E). In addition, at the cancellous bone further from the implant surface, it was noticeable that fewer mineralized trabeculae were found in the OVX group (Figures 5.4E & 5.5E) in contrast to the SHAM (Figures 5.4D and 5.5D) and OVX+SIM groups (Figures. 5.4F & 5.5 F). In contrast, at the cortical zone, the implant surfaces were concealed with established lamellar bone, and no major changes were observed among the three groups.

5.6 DISCUSSION

There have been many studies investigating osseo-integration of dental implants in osteoporotic subjects, but none of them focusing on the posterior maxilla. This study is the first of its kind that specifically evaluates the effect of simvastatin on osseo-integration of dental implants in the posterior maxilla of osteoporotic rats. Simvastatin is a HMG-CoA reductase inhibitor which impedes cholesterol biosynthesis and is commonly utilized as a cholesterol-reducing medication. Lately it has been reported that the liposoluble statin, simvastatin, could up-regulate the expression of BMP-2 mRNA in osteoblasts and, as a consequence, stimulate bone growth[19]. The anabolic influence of statins on bone breakdown has been further documented in numerous animal studies[24, 31]. This fact has produced immense awareness amongst scholars studying conceivable uses of statins in the therapy of bone-related disorders, as well as in dental implantology. A few studies have shown the influence of statins on the osseo-integration of dental implants [26, 27] and these studies have previously demonstrated that simvastatin can enhance osseo-integration in poor quality bone as found in osteoporosis [2]. However, the exact biological mechanisms of this effect were unclear.

In the present study, it was observed that after 28 days, the OVX group had lower BIC and BD in contrast to the SHAM and OVX+SIM groups. This phenomenon suggests that the OVX group may have developed the characteristic alterations of bone turnover seen in osteoporosis (increased bone resorption and reduced bone formation). In the OVX+SIM group, the amount of BIC and BD was not different from the SHAM group. This implies that simvastatin may partially reverse the different turnover attribute of osteoporosis via improvement of osteoblast activity and differentiation, and diminished osteoclastic activity.

At 28 and 56 days, the BFR, BIC and BD of surface-treated implants in both the OVX+SIM and SHAM groups were considerably greater in contrast to the OVX group except for the BFR which was lower at 56 days than at 28 days, indicating that simvastatin stimulates bone growth around titanium implants throughout the initial phases of osseo-integration, and as more bone is in contact with the implant, saturation may have approached its end point. Consequently, this may have resulted in the slowing down of BFR.

Simvastatin treatment had given rise to additional bone cells in the vicinity of the implants in contrast to the untreated OVX group, though exhibiting bone formation features comparable to the SHAM group. The outcomes of BFR, BIC and BD analyses imply that this may be because of improved levels of osteoblastic activity in response to the use of simvastatin.

At 56 days, BIC and BD in the OVX+SIM group were better than in the OVX group but less than in the SHAM group. These effects signify that simvastatin continued to stimulate osteoblastic activity as the recently grown bone near the implant aged.

Interestingly, the BFR, BIC and BD manifested differing developments between days 28 and 56, with BFR decreasing, and BIC and BD growing over time. The relatively fragile relationship between these two markers may suggest the representation of these proteins at various phases of osteoblast differentiation [32].

Several techniques have been employed to investigate bone formation activity.[33, 34] The mineralization of the osteoid matrix represents a crucial stage of this process, and the study of this event by epifluorescence microscopy using fluorochromes may add to the development of the bone regeneration dynamics model.[35] Fluorochromes are fluorescent markers with calcium attraction, and frequently used markers are alizarin, calcein, and oxytetracycline.[36] When several kinds of fluorochromes are introduced into the body at various stages of ossification, they attach to the accessible calcium that is residing in the mineralization areas.[37] Substances linked with bone growth, turnover, and their by-products have been extensively used as indicators of bone metabolism and therefore have a recognized function in the evaluation of osteoporosis and the assessment of treatment results[38]. Calcein green and alizarin are the staining agents that can be incorporated during bone formation [27]. Hence, the fluorescence bone staining agents used in this study were calcein and alizarin red. It has been indicated that calcein

is linked with initial phases of osteogenesis and alizarin is related with successive bone formation.[28] Through the help of filters that grasp particular wavelengths for each fluorochrome, it is possible to observe the mineralized zones in various colours for every phase. Practices employing fluorochromes have been commonly utilized in bone biology studies,[39] and research employing fluorochromes to appraise the dynamics of dental implant osseo-integration is highly valuable.

With the addition of simvastatin not only more mineralized bone was formed but it emerged to become closer to the implant surface as demonstrated with calcein and alizarin staining. This implies that simvastatin had somehow up-regulated the expression of BMB-2 mRNA in osteoblasts to produce more bone [19] and also through the VGF pathway.[40] The order of staining intensity of mineralized bone by the three groups was SHAM > OVX+SIM >OVX and the BFR order was similar, namely: SHAM > OVX+SIM >OVX. However, even in the presence of simvastatin, the BFR of the OVX+SIM was still inferior that of the SHAM group. Therefore, it can be assumed that osteoporotic model used in this study works well in the presence of simvastatin and may have helped to lessen the effect of osteoporosis. The BFR of the OVX+SIM group was better than that of the OVX group, but it was slightly inferior to the SHAM group. This implies that further research is needed on the direct application of simvastatin to implant surface and medicaments needed to improve osseo-integration in osteoporotic subjects.

5.7 CONCLUSION

In conclusion, the present study is the first of its kind that has shown the enhancing effect of simvastatin on osseo-integration of dental implants in the posterior maxilla of osteoporotic rats. It has also demonstrated that new bone formation and mineralization activity are positively correlated with osseo-integration of surface-treated implants in osteoporotic rats treated with simvastatin.

5.8 AKNOWLEDGEMENTS

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Chapter 6: CONCLUSIONS

This thesis has comprehensively addressed issues/problems pertinent to dental implant flapless surgery through six individual chapters. The findings that flapless surgery is a viable treatment option, with high survival and success rates were backed up by clinical and histomorphometric results.

The principal aim of this study was to deliver a better level of understanding, objectivity, and comprehensibility on the merits of dental implant flapless surgical technique. By doing so, the writer has justified hypotheses put forward in section 1.2.3. Hitherto there has been a lack of published literature to substantiate the efficacy and efficiency of flapless technique in the posterior maxilla. To address these shortcomings and to attain the aims, this study has addressed the main objectives as stated in objectives 1.3.2.

Generally, the best scientific method of literature review is meta-analysis and systematic review. Owing to limited published data on this subject, it was only possible to perform a systematic review. This leaves the door open for the former methods as techniques for future research.

Prospective controlled clinical trial would have been ideal; however, resource constraints only allowed this study to use retrospective piloting research to identify important areas for the subsequent chapters. The identification of posterior maxillary low-density bone and its associated causative factors has helped to reveal the importance of using the osteoporotic rat model as the method of choice.

In vivo human evaluation of osseo-integration indices would be an ideal method of choice. Unfortunately, ethical restrictions did not allow this research mode to be used in this study. The results obtained from all the sections of this study have helped to form a convincing notion that “implant flapless surgical technique is an effective and efficient clinical method with relatively high survival rates compared with the flap

counterparts". The implants placed (chapter 4 and 5) soon after molar teeth extraction without raising any flap were considered to be flapless. The osseo-integration indices obtained from rat studies have helped consolidate the above claims. Controlled clinical trial is an important area that deserves future attention.

In a contemporary, democratic, and progressively risk-aware civilization, it is obvious that effective judgement relating to the minimally invasive surgical approach such as "flapless technique" must incorporate both the practical/unbiased/analytical, and the collective/independent/ethical aspects of a particular clinical procedure. Although adopting these complicated attributes, an effective implant flapless technique must warrant results that are acceptable, scientific, universal, and realistic with regard to the pragmatic shortcomings and limitations on procedure.

6.1 SUMMARY OF INNOVATIVE CONTRIBUTIONS

This thesis has made important and fresh academic impacts. These impacts are condensed into separate chapters presented in this thesis. The novel contributions that result from this study are manifold.

Chapter 2- This study has generated a contemporary "systematic review" published in an international peer-reviewed surgical journal. The crucial innovative contribution of the article presented in Chapter 2 is not the measure of the standards themselves but the documentation, depiction, and debate of the subjects, biases, and ambiguities associated with their description. These issues had not been recognised in present quantitative papers. Reflection on these factors has directed attention to a cultivated notion for the design of care standards for implant flapless surgery. These improvements consist in, for instance: the routine use of Cone Beam Computed Tomography as contrasted to conventional two-dimensional radiography as documented in the literature; and the necessity to analyse these aspects of osseo-integration, and not merely rely on diagnostic imaging.

Chapter 3- The "retrospective paper" has demonstrated that the implant flapless surgical technique is a viable and efficient method with a reasonably good survival and success rate. Meaningful contributions were generated with respect to the theoretical identification of the perceptions of implants: flapless surgical technique, survival,

failure, complications, as well as confirming the outcome of the technique through the 10-year retrospective study.

Chapter 4- The results have indicated that osseo-integration (BIC and BD) was inferior in implants following extraction in the posterior maxilla of OVX rats. The main novel contribution here comes with the suggestion that caution needs to be exercised when placing dental implants in osteoporotic subjects, even with the application of commonly used "surface-treated implants" (STIs), as many previous studies employed smooth-surface implants which have become obsolete in the last decade.

Chapter 5- Simvastatin could further assist osseo-integration by boosting osseo-integration indices. There are millions of patients in the world taking simvastatin as a cholesterol-lowering drug. Some of these patients may have had, or be going to have, implant placement in low-density bone in the posterior maxilla. By studying the effects of simvastatin on osseo-integration and its related indices, this study has significantly contributed the additional knowledge that simvastatin can enhance osseo-integration in patients undergoing implant treatment in the posterior maxilla. Furthermore, this study has opened the door for possible future research on simvastatin and STIs.

Collective contribution - The assemblage of work offered in this thesis has tackled the main puzzles associated with performing surgical flapless technique. To the best of the writer's understanding, the obvious value of flapless surgery in conjunction with STIs and simvastatin in the posterior maxilla had not previously been documented in the dental literature, and therefore amounts, in itself, to yet another innovative contribution.

6.2 RESEARCH IMPACT

This study has developed several high-impact results, in particular:

(a) It imparts knowledge about flapless dental implant surgery in an anatomically difficult area, the posterior maxilla, and about how clinicians could best perform the procedure. The analysis of implant complications and failures has helped to identify pitfalls and to consolidate better approaches to this technique.

(b) The retrospective study has reaffirmed that implant flapless surgery is an established and predictable method. With experience and thorough planning, it can deliver a remarkably high survival rate.

(c) Experimental osseo-integration in osteoporotic rats has demonstrated that osteoporosis is not a deterrent to implantation of STIs but rather a sign of the need for caution when using flapless techniques in the posterior maxilla.

(d) The systemic effect of simvastatin on osseo-integration indices has demonstrated the additional benefit of this medication on implantation in low-density bone such as the posterior maxilla.

6.3 FURTHER RESEARCH

This study has laid the groundwork for a more far-reaching research undertaking.

Recommended fields for additional study should embrace:

(a) Future meta-analysis on implant flapless surgery to increase the power of the study.

(b) Long-term prospective controlled clinical trials on flapless technique to enhance the validity and significance of the results.

(c) Research into the effects of various types of STIs on osseo-integration of the posterior maxilla in osteoporotic human subjects instead of rats. This would more realistically reflect the real clinical situation.

(d) Study of simvastatin-coated STIs on osteoporotic subjects to assess its local effects on osseo-integration.

The framework of the six chapters of this thesis, which encompass literature review, clinical study, and histomorphometric evaluation, can be used as a final model of the theory for future research.

6.4 CLOSING REMARKS

Implant flapless surgery is an intricate procedure encompassing multiple technical, anatomical, and theoretical aspects. The bulk of the work reported here does not endeavour to recommend a solution to the questions that appear; instead, this study lays the groundwork for addressing further contemporary issues regarding flapless surgery. Carrying out the research for this PhD thesis has been an enjoyable and rewarding experience. It has also yielded significant contributions to the area of implant flapless surgery and its effects on osteoporotic patients having implants in the posterior maxilla.

Chapter 7: Appendices

7.1 APPENDIX A: PROOF OF PUBLICATION

Journal paper : *"Is flapless implant surgery a viable option in posterior maxilla? A review"* N. Doan, Z. Du, R. Crawford, P. Reher, Y. Xiao, was published in the *International Journal of Oral and Maxillofacial Surgery.* Int. J. Oral Maxillofac. Surg. 2012; 41: 1064-1071.The link to the published article is here: http://eprints.qut.edu.au/. A full text version of the paper is also attached.

Is Flapless Implant Surgery a Viable Option in Posterior Maxilla? A Review

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7.2 APPENDIX B: ADDITIONAL PUBLICATIONS DURING MY PHD CANDIDATURE

7.2.1 Published Journal Paper one:

Application of Autologous Periosteal Cells for the Regeneration of Class III furcation defects in Beagle Dogs

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7.2.2 Published Journal Paper two

Serum bone marker correlation with improved osseointegration in osteoporotic rats treated with Simvastatin

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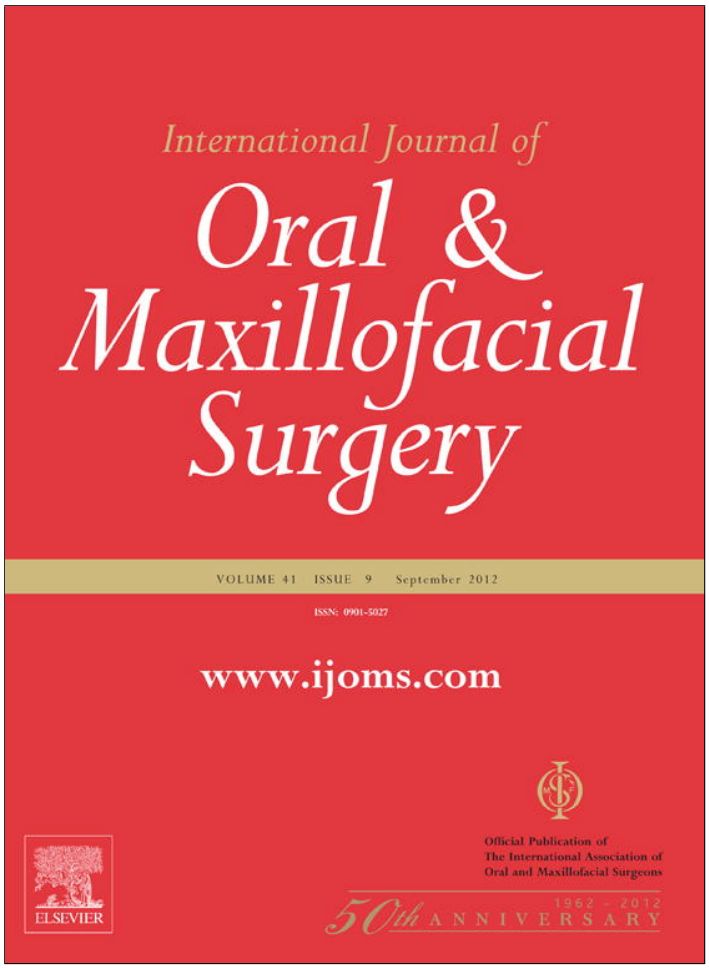

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